UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

Departamento de Ingeniería Química

Effect of Activated Carbon on the Removal of Organic Micropollutants in Activated Sludge and Membrane Bioreactors

Memoria presentada por
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Para optar al grado de Doctor por la
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Informan:

Que la memoria titulada "Effect of Activated Carbon on the Removal of Organic Micropollutants in Activated Sludge and Membrane Bioreactors" que, para optar al grado de Doctor de Ingeniería Química, Programa de Doctorado en Ingeniería Química y Ambiental, presenta Doña Denisse Serrano Palacios, ha sido realizada bajo nuestra inmediata dirección en el Departamento de Ingeniería Química de la Universidad de Santiago de Compostela.

Y para que así conste, firman el presente informe en Santiago de Compostela, Junio de 2011.

Juan M. Lema Rodicio Denisse Serrano Palacios Francisco Omil Prieto

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Objetivos y resumen

La presente Tesis Doctoral se enfoca a la eliminación de microcontaminantes orgánicos, tales como son los compuestos farmacéuticos y de cuidado personal (Pharmaceutical and Personal Care Products, PPCPs) presentes en aguas urbanas en concentraciones a niveles traza, es decir en concentraciones que van desde los ng L^{-1} hasta los μg L^{-1}. En las últimas dos décadas, se ha ido incrementando el conocimiento sobre la presencia de estos compuestos en el medioambiente, gracias fundamentalmente a la aparición de nuevas técnicas analíticas basadas en cromatografía avanzada acoplada a espectrometría de masas (LC-MS o GC-MS), que permiten detectar compuestos en concentraciones muy bajas. Muchos de estos compuestos han sido creados para desarrollar una acción biológica, por lo que el hecho de que sean moléculas biológicamente activas han despertado un creciente interés a lo largo de las últimas décadas debido a la falta de información conclusiva sobre los posibles efectos potenciales sobre el medio ambiente y la salud humana tanto a medio como a largo plazo.

Los PPCPs que se encuentran en el medio ambiente deben su origen a varias fuentes como las prácticas industriales, agrícolas, médicas y prácticas comunes en el hogar (uso de cosméticos, detergentes, etc.). Además, los compuestos farmacéuticos que no son completamente metabolizados en el cuerpo humano y son excretados a través de las heces y orina son otra fuente de contaminación que logran llegar a las aguas residuales. Otra fuente significativa de descarga de fármacos en el medio ambiente son los efluentes de hospitales y los lixiviados generados en los vertederos municipales.

A lo largo de la última década, se ha venido demostrando que las plantas de tratamiento de aguas residuales no son efectivos en la completa eliminación de muchos de los PPCPs presentes en aguas residuales, siendo la eficacia muy variable dependiendo de la configuración y del modo de operación de cada planta. Muchos de estos compuestos son poco biodegradables y se ha determinado su persistencia a lo largo del tiempo (incluso varios años).

A partir de los trabajos publicados se puede concluir que los principales mecanismos de eliminación de PPCPs en una planta de tratamiento de aguas residuales son los siguientes: degradación biológica del compuesto; adsorción y absorción en los sólidos presentes (y posterior gestión final

conjuntamente con los lodos de depuradora); hidrólisis de compuestos conjugados regenerando el compuesto original y stripping (desorción en el licor de mezcla por efecto de la aireación), aunque este mecanismo sólo es relevante para sustancias con volatilidad apreciable. La eliminación global que se pueda alcanzar en una planta determinada se ve acentuada por diversos factores, tales como la actividad microbiana y la naturaleza del contaminante e incluso las condiciones climatológicas locales.

Existen algunos proyectos realizados dentro de nuestro grupo de investigación que han contribuido a mejorar el conocimiento sobre la eliminación de los compuestos farmacéuticos a través de distintas tecnologías aplicadas. De los trabajos realizados se ha concluido que la alternancia de ambientes aerobios y anóxicos permite una mayor eliminación de las sustancias susceptibles de biodegradación y por otra parte se ha observado que un aumento en el tiempo de residencia celular tiene un efecto significativo para lograr una mayor degradación de algunos compuestos.

En este sentido, se ha trabajado con sistemas de reactores basados en el empleo de membranas sumergidas (Reactores Biológicos de Membrana, MBR) que permiten trabajar a altos tiempos de retención celular debido al efecto físico de filtración de la membrana (normalmente de Micro o Ultrafiltración). Otra configuración que ha sido probada para la eliminación de PPCPs, es la aplicación de reactores biológicos de membrana híbridos, que permite disponer de biomasa tanto en suspensión como en forma de biopelícula sobre un relleno presente en el sistema, incrementando así la disponibilidad de biomasa y el potencial de biodegradación, sin que se tenga lugar un incremento significativo en la cantidad de lodo producido.

Existen otras propuestas que consisten en el uso de tratamientos terciarios como la oxidación con ozono, que ha demostrado una gran eficacia de eliminación de determinados PPCPs, así como el uso de coagulantes y floculantes. Una alternativa especialmente efectiva es el uso de carbón activo ya sea granular (GAC) o en polvo (PAC), con el que pueden conseguir eliminaciones muy importantes para un gran número de estos compuestos presentes a niveles traza. Sin embargo, existen algunas investigaciones que han demostrado que cuando hay presencia de materia orgánica, decae la eficacia, ya que tiene lugar una competición entre macro y microcontaminantes por los centros activos del adsorbente.

En base a todo lo anteriormente citado, el objetivo global de esta Tesis Doctoral está enfocado a la eliminación de PPCPs, especialmente los de carácter más recalcitrante, utilizando distintas condiciones y estrategias de operación sobre los sistemas convencionales de lodos activos. La lista de compuestos utilizada en este trabajo comprende un grupo representativo de PPCPs que pertenecen a distintos grupos terapéuticos (antibióticos, antidepresivos, antiinflamatorios, tranquilizantes y antiepilépticos), fragancias usadas en la formulación de cosméticos y la hormona sintética etinilestradiol (EE2).

Por lo tanto, los objetivos específicos de esta Tesis son:

• Estudiar los procesos de adsorción de los PPCPs seleccionados sobre lodos o sobre diversos tipos de carbón activado. Se determinarán las isotermas de adsorción en ensayos en discontinuo, considerando distintas condiciones ambientales. En este mismo sentido, se estudiará un modelo de tres compartimentos, con el propósito de comprender mejor los procesos de adsorción. Se aplicarán balances de materia tomando en cuenta las concentraciones de los PPCPs en la fase líquida y sólida (carbón activo y lodos).

• Evaluar la eliminación de los PPCPs seleccionados, añadidos en una alimentación con características de una agua residual urbana de carga media, en un sistema aerobio híbrido (HAS), una alternativa innovadora en la cual, además de biomasa en suspensión, se desarrolla una biopelícula gracias a la presencia de un soporte en el tanque de aireación. De esta manera, se comparará la eficacia obtenida con este sistema con un reactor convencional de lodos activos.

• Evaluar la viabilidad de usar carbón activo granular (GAC) directamente en el tanque de aeración de un sistema convencional de lodos activos, así como también la adición de un coagulante ($FeCl_3$), para eliminar los PPCPs seleccionados.

• Investigar la eliminación de los compuestos farmacéuticos seleccionados contenidos en un agua residual urbana sintética de carga alta, usando un biorreactor secuencial de membranas. Además, se evaluará la adición de carbón activado en polvo (PAC) en el tanque de aireación del reactor como una estrategia más para incrementar la eliminación de los compuestos farmacéuticos.

Los resultados experimentales y la discusión se presentan divididos en seis capítulos.

En el **capítulo 1**, se presenta una revisión bibliográfica actualizada de los estudios efectuados hasta la fecha sobre la eliminación de PPCPs en las aguas residuales haciendo especial hincapié en la utilización de nuevas estrategias como el uso de birreactores de membranas, sistemas híbridos de biopelícula, así como el uso de aditivos tales como el carbón activo ya sea en polvo (PAC) o granular (GAC) en el tanque de aireación. Asimismo en este capítulo, se hace una revisión histórica de la evolución del proceso convencional de lodos activos. También se plantea la problemática actual sobre la presencia de microcontaminantes orgánicos, sus posibles efectos sobre la biota acuática y las fuentes y descargas de estos contaminantes en las aguas. Se indican, por último, los PPCPs considerados en esta Tesis, indicando sus características físico-químicas y los mecanismos que afectan su eliminación en las plantas de tratamiento de aguas residuales.

En el **capítulo 2**, se presentan los materiales y métodos utilizados a lo largo de los experimentos realizados en los capítulos posteriores: los métodos empleados para analizar las propiedades en las fases líquida y sólida, así como la caracterización física y microbiológica de la biomasa. Se determinaros las poblaciones microbianas que se encuentran típicamente en los sistemas de tratamientos de aguas residuales mediante el uso de la técnica de hibridación *in situ* con sondas fluorescentes (FISH). Además, se anexa una tabla que describe las distintas sondas aplicadas para la detección de bacterias, así como también el árbol filogenético que muestra la aplicación de cada sonda.

Finalmente, se detallan los métodos analíticos empleados para determinar los PPCPs. La lista de microcontaminantes orgánicos empleados en este trabajo incluye: fragancias policíclicas (galaxolide, tonalide y celestolide), antiepiléptico (carbamazepina), tranquilizante (diazepam), anti-inflamatorios (ibuprofeno, naproxeno y diclofenaco), antidepresivo (fluoxetina), la hormona sintética 17α-etinilestradiol y antibióticos (roxitromicina, trimetoprim y eritromicina).

En el **capítulo 3**, se aplica un modelo de distribución del microcontaminante en una matriz de lodos activos. Este modelo considera al licor de mezcla como un sistema de tres compartimentos, donde el microcontaminante puede encontrarse en un estado libre, adsorbido a las partículas ó adsorbido a la materia disuelta y coloidal que se encuentra en la matriz. Este modelo

permite comprender la afinidad que el compuesto pueda tener hacia cualquiera de las tres fases, a través de la determinación de dos coeficientes de equilibrio K_{part} y K_{DCM}. Este modelo ha sido aplicado para el estudio y distribución de la hormona sintética 17α-etinilestradiol (EE2) utilizando un rango de variación de Materia Disuelta y Coloidal (DCM) de 1.5 a 5 mg L^{-1}. Los resultados experimentales permiten concluir que la presencia de DCM para este compuesto no influye de forma significativa en su distribución, por lo que la mayor afinidad de este compuesto se encuentra hacia las partículas. Este trabajo fue llevado a cabo en el Laboratoire de Biotechnologie de l´Environement (L.B.E.), unidad de investigación del Institut National de la Recherche Agronomique (INRA) en Narbona, Francia.

En el **capítulo 4** se estudia la influencia de una biopelícula desarrollada en un sistema hibrido a escala laboratorio sobre la eliminación de los PPCPs. Los resultados obtenidos usando este sistema son comparados con los de un sistema convencional de lodos activos. El sistema híbrido (HAS) se desarrolla a partir de la incorporación de un soporte sintético (Kaldnes) al tanque de aireación del sistema de lodos activos. Sobre la biopelícula se puede desarrollar una biomasa con bacterias que requieren un tiempo de residencia celular muy alto para su crecimiento, como las bacterias nitrificantes, mientras que otras pueden permanecer como biomasa en suspensión. No se logra observar un efecto significativo en la eliminación de los compuestos más recalcitrantes probablemente debido al poco crecimiento de la biopelícula sobre el soporte debido al tiempo limitado de operación (60 d). Sin embargo, para otros compuestos como el ibuprofeno, naproxeno y las fragancias, se alcanzan porcentajes de eliminación superiores al 90% en el sistema híbrido. A pesar del corto tiempo de operación del reactor HAS, parece que la presencia de la biopelícula permite mejorar la eliminación de algunos microcontaminantes.

En el **capítulo 5** se estudia la eliminación de los PPCPs utilizando tres sistemas convencionales de lodos activos, evaluando el efecto del uso de aditivos como coagulante (cloruro de hierro) o carbón activo en forma granular (GAC) directamente en el tanque de aireación de los reactores. Los resultados demuestran que el efecto del coagulante es casi despreciable en la eliminación de los PPCPs y que la adición del GAC provoca una eliminación de hasta un 40% para compuestos como carbamazepina y diazepam que tienen un carácter recalcitrante y de hasta un 85% para diclofenaco. Cabe mencionar que las mejores eficacias de eliminación se obtuvieron cuando se añadieron las mayores dosificaciones de GAC al sistema.

Además, se llevan a cabo ensayos en discontinuo para determinar el efecto del carbón activo sobre la afinidad y eliminación de los PPCPs. Dichos experimentos determinan que la mayor afinidad del GAC es con aquellas sustancias con carácter recalcitrante como la carbamazepina, el diazepam y el diclofenaco y por el contrario, aquellos compuestos mas lipofílicos, como las fragancias (galaxolide, tonalide y celestolide), muestran una menor afinidad. Por lo tanto, la principal contribución de este capítulo es la viabilidad del uso del carbón activo granular en reactores convencionales de lodos activos como una estrategia para incrementar la eliminación de PPCPs recalcitrantes.

En el **capítulo 6**, se enfatiza el efecto de carbón activo en polvo (PAC) sobre la eliminación de PPCPs en un reactor secuencial (SBR) con una cámara de membrana de microfiltración. El sistema en conjunto es denominado Sequential Membrane Biological Reactor (SMBR). Se añade una única dosis de carbón activo en polvo dentro del tanque de aireación. Mediante este sistema se logra reducir no sólo el contenido en materia orgánica (>95%) y nutrientes como $N\text{-}NH_4^+$ and $P\text{-}PO_4^{-3}$ (>80%) sino que se consiguen altos rendimientos (90-99%) en la eliminación de PPCPs presentes a niveles traza tales como antiinflamatorios (p.ej. ibuprofeno, naproxeno y diclofenaco), antibióticos (p.ej. trimetoprim, roxitromicina y eritromicina), tranquilizantes (diazepam), antiepilépticos (carbamazepina), antidepresivos (fluoxetina).

La adición del PAC al reactor permite eliminar las sustancias con carácter persistente tales como carbamazepina, diazepam, diclofenaco y trimetoprim, cuyas eficacias de eliminación pasan de ser menores al 10% en ausencia de PAC hasta valores del 95-99% en presencia del adsorbente. También se consigue incrementar significativamente la eliminación de otros compuestos que en ausencia de PAC presentan eliminaciones moderadas (en el rango del 40 al 70%) para estar por encima del 98% tras la adición del carbón activo (caso de los antibióticos roxitromicina y eritromicina y del antidepresivo fluoxetina). A diferencia de estos compuestos, la eliminación de las fragancias consideradas así como de los antiinflamatorios ibuprofeno y naproxeno no se ve afectada por la adición de PAC, debido a que la absorción y/o la biodegradación son los principales mecanismos de eliminación para estos compuestos.

Con los trabajos realizados en esta Tesis Doctoral se ha conseguido una información valiosa sobre la eliminación de compuestos cosméticos y farmacéuticos especialmente de aquellos con carácter más persistente en

aguas residuales urbanas. Además, la implementación de diferentes estrategias como el uso del carbón activo como adsorbente, ya sea en forma granular (GAC) o en polvo (PAC), dentro del tanque de aireación de los sistemas convencionales de lodos activos han destacado por su mejora en cuanto a la eficiencia global del sistema así como de la eliminación de los PPCPs.

Obxectivos e Resumo

A presente Tese Doutoral enfócase á eliminación de microcontaminantes orgánicos, tales como son os compostos farmacéuticos e de coidado persoal (Pharmaceutical and Persoal Care Products, PPCPs) presentes en augas urbanas en concentracións a niveis traza, é dicir en concentracións que van desde os ng L^{-1} até os µg L^{-1}. Nas últimas dúas décadas, foise incrementando o coñecemento sobre a presenza destes compostos no medio ambiente, grazas fundamentalmente á aparición de novas técnicas analíticas baseadas en cromatografía avanzada axustada a espectrometría de masas (LC-MS ou GC-MS), que permiten detectar compostos en concentracións moi baixas. Moitos destes compostos foron creados para desenvolver unha acción biolóxica, polo que o feito de que sexan moléculas bioloxicamente activas espertou un crecente interese ao longo das últimas décadas debido á falta de información conclusiva sobre os posibles efectos potenciais sobre o medio ambiente e a saúde humana tanto a medio como a longo prazo.

Os PPCPs que se atopan no medio ambiente deben a súa orixe a varias fontes como as prácticas industriais, agrícolas, médicas e prácticas comúns no fogar (uso de cosméticos, deterxentes, etc). Ademais, os compostos farmacéuticos que non son completamente metabolizados no corpo humano e son excretados a través das feces e ouriños son outra fonte de contaminación que logra chegar ás augas residuais. Outra fonte significativa de descarga de fármacos no medio ambiente son os efluentes de hospitais e os lixiviados xerados nos vertedoiros municipais.

Ao longo da última década, veuse demostrando que as plantas de tratamento de augas residuais non son efectivas na completa eliminación de moitos dos PPCPs presentes nas augas residuais, sendo a eficacia moi variable dependendo da configuración e do modo de operación de cada planta. Moitos destes compostos son pouco biodegradables e determinouse a súa persistencia ao longo do tempo (incluso varios anos).

A partir dos traballos publicados pódese concluír que os principais mecanismos de eliminación de PPCPs nunha planta de tratamento de augas residuais son os seguintes: degradación biolóxica do composto; absorción e adsorción nos sólidos presentes (e posterior xestión final conxuntamente cos lodos de depuradora); hidrólise de compostos conxugados rexenerando o

composto orixinal e stripping (desorción no licor de mestura por efecto da aireación), aínda que este mecanismo só é relevante para sustancias con volatilidade apreciable. A eliminación global que se poida alcanzar nunha planta determinada vese acentuada por diversos factores, tales como a actividade microbiana e a natureza do contaminante e mesmo as condicións climatolóxicas locais.

Existen algúns proxectos realizados dentro do noso grupo de investigación que contribuíron a mellorar o coñecemento sobre a eliminación dos compostos farmacéuticos a través de distintas tecnoloxías aplicadas. Dos traballos realizados concluíuse que a alternancia de ambientes aerobios e anóxicos permite unha maior eliminación das sustancias susceptibles de biodegradación e por outra banda observouse que un aumento no tempo de residencia celular ten un efecto significativo para lograr unha maior degradación dalgúns compostos.

Neste sentido, traballouse con sistemas de reactores baseados no emprego de membranas mergulladas (Reactores Biolóxicos de Membrana, MBR) que permiten traballar a altos tempos de retención celular debido ao efecto físico de filtración da membrana (normalmente de Micro ou Ultrafiltración). Outra configuración que foi probada para a eliminación de PPCPs, é a aplicación de reactores biolóxicos de membrana híbridos, que permite dispor de biomasa tanto en suspensión como en forma de biopelícula sobre un recheo presente no sistema, incrementando así a dispoñibilidade de biomasa e o potencial de biodegradación, sen que teña lugar un incremento significativo na cantidade de lodo producido.

Existen outras propostas que consisten no uso de tratamentos terciarios como a oxidación con ozono, que demostrou unha gran eficacia de eliminación de determinados PPCPs, así como o uso de coagulantes e floculantes. Unha alternativa especialmente efectiva é o uso de carbón activo xa sexa granular (GAC) ou en po (PAC), co que poden conseguirse eliminacións moi importantes para un gran número destes compostos presentes a niveis traza. Con todo, existen algunhas investigacións que demostraron que cando hai presenza de materia orgánica, decae a eficacia , xa que ten lugar unha competición entre macro e microcontaminantes polos centros activos do adsorbente.

En base a todo o anteriormente citado, o obxectivo global desta Tese Doutoral está enfocado á eliminación de PPCPs, especialmente os de carácter máis recalcitrante, utilizando distintas condicións e estratexias de operación

sobre os sistemas convencionais de lodos activos. A lista de compostos utilizada neste traballo comprende un grupo representativo de PPCPs que pertencen a distintos grupos terapéuticos (antibióticos, antidepresivos, antiinflamatorios, tranquilizantes e antiepilépticos), fragrancias usadas na formulación de cosméticos e a hormona sintética etinilestradiol (EE2).

Por tanto, os obxectivos específicos desta Tese son:

• Estudar os procesos de adsorción dos PPCPs seleccionados sobre lodos ou sobre diversos tipos de carbón activado. Determinaranse as isotermas de adsorción en ensaios en descontinuo, considerando distintas condicións ambientais. Neste mesmo sentido, estudarase un modelo de tres compartimentos, co propósito de comprender mellor os procesos de adsorción. Aplicaranse balances de materia tomando en conta as concentracións dos PPCPs na fase líquida e sólida (carbón activo e lodos).

• Avaliar a eliminación dos PPCPs seleccionados, engadidos nunha alimentación con características dunha auga residual urbana de carga media, nun sistema aerobio híbrido (HAS), unha alternativa innovadora na cal, ademais de biomasa en suspensión, desenvólvese unha biopelícula grazas á presenza dun soporte no tanque de aireación. Desta maneira, compararase a eficacia obtida con este sistema cun reactor convencional de lodos activos.

• Avaliar a viabilidade de usar carbón activo granular (GAC) directamente no tanque de aireación dun sistema convencional de lodos activos, así como tamén a adición dun coagulante ($FeCl_3$), para eliminar os PPCPs seleccionados.

• Investigar a eliminación dos compostos farmacéuticos seleccionados contidos nunha auga residual urbana sintética de carga alta, usando un biorreactor secuencial de membranas. Ademais, avaliarase a adición de carbón activado en po (PAC) no tanque de aireación do reactor como unha estratexia máis para incrementar a eliminación dos compostos farmacéuticos.

Os resultados experimentais e a discusión preséntanse divididos en seis capítulos.

No capítulo 1, preséntase unha revisión bibliográfica actualizada dos estudos efectuados até a data sobre a eliminación de PPCPs nas augas residuais facendo especial fincapé na utilización de novas estratexias como o uso de biorreactores de membranas, sistemas híbridos de biopelícula, así

como o uso de aditivos tales como o carbón activo xa sexa en po (PAC) ou granular (GAC) no tanque de aireación. Así mesmo neste capítulo, faise unha revisión histórica da evolución do proceso convencional de lodos activos. Tamén se expón a problemática actual sobre a presenza de microcontaminantes orgánicos, os seus posibles efectos sobre a biota acuática e as fontes e descargas destes contaminantes nas augas. Indícanse, por último, os PPCPs considerados nesta Tese, indicando as súas características físico-químicas e os mecanismos que afectan a súa eliminación nas plantas de tratamento de augas residuais.

No capítulo 2, preséntanse os materiais e métodos utilizados ao longo dos experimentos realizados nos capítulos posteriores: os métodos empregados para analizar as propiedades nas fases líquida e sólida, así como a caracterización física e microbiolóxica da biomasa. Determinaranse as poboacións microbianas que se atopan tipicamente nos sistemas de tratamento de augas residuais mediante o uso da técnica de hibridación in situ con sondas fluorescentes (FISH). Ademais, anéxase unha táboa que describe as distintas sondas aplicadas para a detección de bacterias, así como tamén a árbore filoxenética que mostra a aplicación de cada sonda.

Finalmente, detállanse os métodos analíticos empregados para determinar os PPCPs. A lista de microcontaminantes orgánicos empregados neste traballo inclúe: fragrancias policíclicas (galaxolide, tonalide e celestolide), antiepiléptico (carbamazepina), tranquilizante (diazepam), anti-inflamatorios (ibuprofeno, naproxeno e diclofenaco), antidepresivo (fluoxetina), a hormona sintética 17α-etinilestradiol e antibióticos (roxitromicina, trimetoprim e eritromicina).

No capítulo 3, aplícase un modelo de distribución do microcontaminante nunha matriz de lodos activos. Este modelo considera ao licor de mestura como un sistema de tres compartimentos, onde o microcontaminante pode atoparse nun estado libre, adsorbido ás partículas ou adsorbido á materia disolta e coloidal que se atopa na matriz. Este modelo permite comprender a afinidade que o composto poida ter cara a calquera do tres fases, a través da determinación de dous coeficientes de equilibrio K_{part} e K_{DCM}. Este modelo foi aplicado para o estudo e distribución da hormona sintética 17α-etinilestradiol (EE2) utilizando un rango de variación de Materia Disolta e Coloidal (DCM) de 1.5 a 5 mg L^{-1}. Os resultados experimentais permiten concluír que a presenza de DCM para este composto non inflúe de forma significativa na súa distribución, polo que a maior afinidade deste composto

atópase cara ás partículas. Este traballo foi levado a cabo no Laboratoire de Biotechnologie de l´Environnement (L.B.E.), unidade de investigación do Institut National da Recherche Agronomique (INRA) en Narbona, Francia.

No capítulo 4 estúdase a influencia dunha biopelícula desenvolvida nun sistema hibrido a escala laboratorio sobre a eliminación dos PPCPs. Os resultados obtidos usando este sistema son comparados cos dun sistema convencional de lodos activos. O sistema híbrido (HAS) desenvólvese a partir da incorporación dun soporte sintético (Kaldnes) ao tanque de aireación do sistema de lodos activos. Sobre a biopelícula pódese desenvolver unha biomasa con bacterias que requiren un tempo de residencia celular moi alto para o seu crecemento, como as bacterias nitrificantes, mentres que outras poden permanecer como biomasa en suspensión. Non se logra observar un efecto significativo na eliminación dos compostos máis recalcitrantes probablemente debido ao pouco crecemento da biopelícula sobre o soporte polo tempo limitado de operación (60 d). Con todo, para outros compostos como o ibuprofeno, naproxeno e as fragrancias, alcánzanse porcentaxes de eliminación superiores ao 90% no sistema híbrido. A pesar do curto tempo de operación do reactor HAS, parece que a presenza da biopelícula permite mellorar a eliminación dalgúns microcontaminantes.

No capítulo 5 estúdase a eliminación dos PPCPs utilizando tres sistemas convencionais de lodos activos, avaliando o efecto do uso de aditivos como coagulante (cloruro de ferro) ou carbón activo en forma granular (GAC) directamente no tanque de aireación dos reactores. Os resultados demostran que o efecto do coagulante é case desprezable na eliminación dos PPCPs e que a adición do GAC provoca unha eliminación de até un 40% para compostos como carbamazepina e diazepam que teñen un carácter recalcitrante e de até un 85% para diclofenaco. Cabe mencionar que as mellores eficacias de eliminación se obtiveron cando se engadiron as maiores doses de GAC ao sistema.

Ademais, leváronse a cabo ensaios en descontinuo para determinar o efecto do carbón activo sobre a afinidade e eliminación dos PPCPs. Devanditos experimentos determinan que a maior afinidade do GAC é con aquelas sustancias con carácter recalcitrante como a carbamazepina, o diazepam e o diclofenaco e pola contra, aqueles compostos mais lipofílicos, como as fragrancias (galaxolide, tonalide e celestolide), mostran unha menor afinidade. Por tanto, a principal contribución deste capítulo é a viabilidade do uso do carbón activo granular en reactores convencionais de lodos activos

como unha estratexia para incrementar a eliminación de PPCPs recalcitrantes.

No capítulo 6, saliéntase o efecto do carbón activo en po (PAC) sobre a eliminación de PPCPs nun reactor secuencial (SBR) cunha cámara de membrana de microfiltración. O sistema en conxunto é denominado Sequential Membrane Biological Reactor (SMBR). Engádese unha única dose de carbón activo en po dentro do tanque de aireación. Mediante este sistema lógrase reducir non só o contido en materia orgánica (>95%) e nutrientes como $N\text{-}NH_4^+$ e $P\text{-}PO_4^{-3}$ (>80%) senón que se conseguen altos rendementos (90-99%) na eliminación de PPCPs presentes a niveis traza tales como antiinflamatorios (p.ex. ibuprofeno, naproxeno e diclofenaco), antibióticos (p.ex. trimetoprim, roxitromicina e eritromicina), tranquilizantes (diazepam), antiepilépticos (carbamazepina), e antidepresivos (fluoxetina).

A adición do PAC ao reactor permite eliminar as sustancias con carácter persistente tales como carbamazepina, diazepam, diclofenaco e trimetoprim, cuxas eficacias de eliminación pasan de ser menores ao 10% en ausencia de PAC até valores do 95-99% en presenza do adsorbente. Tamén se consegue incrementar significativamente a eliminación doutros compostos que en ausencia de PAC presentan eliminacións moderadas (no rango do 40 ao 70%) para estar por encima do 98% trala adición do carbón activo (caso dos antibióticos roxitromicina e eritromicina e do antidepresivo fluoxetina). A diferenza destes compostos, a eliminación das fragrancias consideradas así como dos antiinflamatorios ibuprofeno e naproxeno non se ve afectada pola adición de PAC, debido a que a absorción e/ou a biodegradación son os principais mecanismos de eliminación para estes compostos.

Cos traballos realizados nesta Tese Doutoral conseguiuse unha información valiosa sobre a eliminación de compostos cosméticos e farmacéuticos especialmente daqueles con carácter máis persistente en augas residuais urbanas. Ademais, a implantación de diferentes estratexias como o uso do carbón activo como adsorbente, xa sexa en forma granular (GAC) ou en po (PAC), dentro do tanque de aireación dos sistemas convencionais de lodos activos destacaron pola súa mellora en canto á eficiencia global do sistema así como da eliminación dos PPCPs.

Objectives and summary

This Doctoral Thesis is focused on the removal of organic micropollutants, such as Pharmaceutical and Personal Care Products (PPCPs) present in sewage in concentrations from ng L^{-1} to µg L^{-1}. In the last two decades the knowledge about the occurrence of these compounds in the environment has increased, due to the advance in analytical techniques based on chromatography with Mass Spectrometry (LC-MS or GC-MS) that allow the detection and quantification of compounds at trace levels. Most of the PPCPs are biologically active compounds that have been designed to alter biological systems. Therefore, the lack of information about their potential effects on the environment and human health is one of the reasons to study and evaluate these compounds.

The principal sources of PPCPs in the environment are either industrial, agricultural, medical manufactured or common household practices (use of cosmetics, detergents, etc.). Moreover, pharmaceuticals which are not completely metabolised in the human body are excreted with the urine and feces and can reach urban wastewater. Additionally, other significant sources of pharmaceutical discharge in the environment are the effluents from hospitals and leachates generated from municipal landfills.

Over the last decade it was shown that conventional treatment plants are not effectives to remove many of PPCPs present in wastewater and also that removal efficiencies depends on configuration and operation mode of each plant. Many of these compounds are persistent to biodegradation and their presence in the environment has been detected a long time.

The main removal mechanisms of PPCPs observed in treatment plants are: biological degradation, sorption onto sludge, hydrolysis of conjugates (that can form again the parent compound) and volatilisation or stripping. The importance of the different mechanisms depends on a range of factors, such as weather conditions, microbial activity or the nature of the pollutant.

According to different projects carried out in the research group at the University of Santiago de Compostela, it was concluded that applying aerobic and anoxic environments allows high removal efficiencies of some compounds with low susceptibility to biodegradation. Besides, sludge retention time exerts a significant effect on the achievement of a higher

biodegradation for some compounds. In this sense, the use of Submerged Membrane Bioreactors (MBRs) working at high sludge retention combined with the physical effect of the membrane filtration was studied. In addition, other configurations were tested for PPCPs removal such as Hybrid Membrane Biological Reactor (HMBR), which used biomass both in suspension and a biofilm growing on the surface of biocarrier. This system increases the availability of biomass and capacity of biodegradation without a significant increase in sludge production. Other alternatives include the use of tertiary treatment such as oxidation with ozone, which has proven to be highly effective in the removal of certain PPCPs and the use of coagulants and flocculation processes and activated carbon either in granular (GAC) or powdered (PAC) form. However, there are reports showing that the efficiency of pharmaceutical compounds removal by the activated carbon can decrease in presence of organic matter due to the competition between macro and micropollutants to the active centers of the adsorbent.

This Doctoral Thesis aims at studying removal of PPCPs from sewage, mainly those with a more persistent behaviour, under different operational conditions and operational strategies applied in the conventional activated sludge systems. The list of PPCPs included in this research comprises pharmaceuticals from five different therapeutic classes (antibiotics, anti-depressants, anti-inflammatory drugs, tranquilizers and anti-epileptics), the synthetic hormone ethinylestradiol (EE2) and three polycyclic musk fragrances.

The specific objectives of this thesis were:

• To study the adsorption process of selected PPCPs either onto sludge or onto activated carbon. Batch adsorption isotherms were determined considering different environmental conditions. In this way, a three-compartment matrix model was studied for a better understanding of the adsorption process. Mass balances were applied taking into account PPCPs concentrations both in liquid and solid phases (activated carbon and sludge).

• To evaluate the removal of selected Pharmaceutical and Personal Care Products spiked in a synthetic feeding simulating the typical composition of medium-strength sewage, in a Hybrid Aerobic System (HAS), with biofilm support, and to compare their results with the obtained in a Conventional Activated Sludge (CAS) system.

• To evaluate the effect of Granular Activated Carbon (GAC) in the aeration tank of Conventional Activated Sludge (CAS) reactors, studying also the influence of the effect of coagulants such as Ferric Chloride ($FeCl_3$) for removal of PPCPs.

• To assess the removal of selected pharmaceutical micropollutants present in high-strength synthetic sewage, using a Sequential Membrane Batch Reactor (SMBR) with a microfiltration membrane. Moreover, the addition of PAC directly into the aeration tank was also assessed as a tool to enhance the removal of the most recalcitrant compounds.

The experimental results and discussions are detailed in six chapters:

In **chapter 1**, an updated revision of the literature referring to the studies on PPCPs removal from wastewater is presented. Special attention has been paid to the use of new strategies such as membrane bioreactors, hybrid systems (biofilm + suspended biomass) and the use of additives such as activated carbon either in powdered (PAC) or granular (GAC) form in the aeration tank. In addition, a revision on the evolution of activated sludge treatment plants is studied. Moreover, a discussion about the problems due to the occurrence of organic micropollutants in the environment, their possible toxic effects over aquatic organisms and their sources and fates in water are presented. Finally, a list of the compounds used in this Thesis including, their physico-chemical characteristics and their main removal mechanisms in wastewater treatment plants are described.

In **chapter 2**, materials and methods employed in the experimental work in subsequent chapters are explained: Protocols for analysis in aqueous and solid phase, physical and microbiological characteristics of the biomass are included. Microbial populations typically found in biological wastewater treatment were followed by Fluorescence In Situ Hybridization (FISH). Also, an extensive table describing the gene probes applied for the detection of bacteria and a phylogenetic tree showing the application of the probes are indicated.

Finally, the analytical methods for PPCPs determination are detailed. The organic micropollutants employed in this work including polycyclic musk fragrances (galaxolide, tonalide and celestolide), anti-epileptic (carbamazepine), tranquilizer (diazepam), anti-inflammatory drugs (ibuprofen, naproxen and diclofenac), anti-depressant (fluoxetine), estrogen

(17α-ethinylestradiol) and antibiotics (roxythromycin, trimethroprim and erythromicyn).

In **chapter 3**, a model of micropollutant distribution in an activated sludge matrix is studied. This model considers the sludge as a three compartment matrix, in which the pollutant remains in a free state, sorbed to particles or sorbed to dissolved and colloidal matter in the matrix. This approach permits to gain a better knowledge about the affinity of the pollutant for any of these phases, through the determination of two equilibrium constants: K_{part} and K_{DCM}. The model has been applied to study the distribution of the synthetic hormone ethinylestradiol (EE2) using Dissolved and Colloidal Matter (DCM) in the range from 1.5 to 5 mg L^{-1}. The experimental results allow concluding that the presence of DCM did not influence significantly EE2 distribution, being most of ethinylestradiol associated to particulate matter. This work was carried out in the Laboratory of Environmental Biotechnology (LBE), a research unit of the French National Institute for Agricultural Research (INRA) in Narbonne, France.

In **Chapter 4**, the influence of the biofilm developed in a laboratory-scale hybrid system on removal of PPCPs is presented. The results obtained using this system are compared with the results obtained in a conventional activate sludge system. The Hybrid Aerobic System (HAS) includes a synthetic support (Kaldnes) in the aeration tank. It is expected that microorganisms requiring higher sludge ages, such as nitrifying biomass, develop onto the support surface while other microorganisms remain as free biomass. The results show no significant effects on the more persistent compounds removals, such as carbamazepine, diazepam and diclofenac, probably due to the limited development of biofilm on the support. However, for other compounds, such as ibuprofen, naproxen and musk fragrances removals up to 90 % were achieved in HAS reactor. Despite of the short operation time of HAS reactor, it seems that the developed biofilm permitted to enhance the removal of some micropollutants.

In **chapter 5**, removal of PPCPs in three activated sludge reactors was studied, assessing the effect of adding a coagulant (ferric chloride) or granular activated carbon (GAC) to the aeration tank. The results indicate that the influence of coagulant addition on PPCPs was negligible, while the addition of GAC enhanced the removal up to 40% for recalcitrant compounds such as carbamazepine and diazepam and 85% for diclofenac. The best removal efficiencies were achieved at higher GAC concentrations.

In addition, batch experiments were carried out in order to determine the effect of activated carbon on affinity and further removal of PPCPs. According to these experiments, the results show that the higher GAC affinity is to compounds with recalcitrant characteristics, such as the anti-epileptic carbamazepine, the tranquilizer diazepam, and the anti-inflammatory diclofenac, whereas compounds with lipophilic characteristics like musk fragrances (galaxolide, tonalide and celestolide) have low affinity. The results suggest the addition of activated carbon in the conventional activated sludge reactors as a strategy to increase removal of some recalcitrant PPCPs.

Chapter 6 emphasizes the effect of powdered activated carbon (PAC) on PPCPs removal in Sequential Membrane Biological Reactor (SMBR). A single dose of PAC was added to the aeration tank. This system proved to achieve not only high reduction of organic matter (>95%) and macronutrients such as $N\text{-}NH_4^+$ and $P\text{-}PO_4^{-3}$ (>80%), but also a high removal efficiencies (90-99%) of PPCPs, that were in trace levels such as anti-inflammatory (i.e. ibuprofen, naproxen and diclofenac) and anti-depressants (fluoxetine).

Furthermore, the addition of PAC to the reactor permitted the elimination of persistent compounds such as carbamazepine, diazepam, diclofenac and trimethoprim, which removal efficiencies increased from 10 to 95-99%. Also, in the case of compounds with moderate eliminations rates (40 to 70%) (such as roxithromycin, erythromycin and fluoxetine) the addition of PAC made possible to achieve up to a 98% removal. However, the effect of powdered activated carbon on the removal of some compounds such a musk fragrances and the anti-inflammatory drugs, ibuprofen and naproxen was negligible, due to the fact that absorption and/or biodegradation are the main removal mechanisms for these compounds.

According to the research carried out in this Thesis, valuable information on pharmaceutical and cosmetic compounds removals is reported, particularly of those compounds with persistent characteristics in sewage. Moreover, the implementation of different strategies such as activated carbon either in powdered (PAC) or granular (GAC) form in the aeration tanks of conventional activated sludge systems are highlighted to enhance the performance of the system and the PPCPs removal.

Chapter 1

Introduction

1.1. EMERGING MICROPOLLUTANTS AS A NEW CHALLENGE

In the last decades a growing attention has been given to the occurrence of micropollutants in the environment due to the lack of conclusive information about the possible impact on the human health and ecosystem (Ashton et al., 2004; Fono et al., 2006; Vanderford and Snyder, 2006). Since the 70s, occurrence of pharmaceutical compounds in municipal sewage was reported in USA (Garrison et al., 1976). In Ontario and western Canada, several studies have reported the occurrence of acidic and neutral drugs in municipal sewage and surface water, showing that the majority of these compounds are removed partially or are not at all by conventional sewage treatment processes (Rogers et al., 1986). A wide range of pharmaceutical compounds were detected in UK rivers and estuaries, being established as the main source the STPs effluents discharges (Hilton and Thomas, 2003). In Germany, the occurrence of drugs in STP effluents, surface water, groundwater and drinking water has been deeply studied (Ternes and Joss, 2006). Furthermore, in the last two decades the knowledge on the occurrence of these compounds in the environment across the world has experienced an important development thanks to the advance in analytical techniques based on chromatography coupled with Mass Spectrometry (LC-MS or GC-MS) that permit the detection and quantification of compounds at trace levels, μg L^{-1} or ng L^{-1}.

"Pharmaceutical and Personal Care Products (PPCPs)" concept covers a wide variety of substances different in structure, aqueous solubility, repartition octanol-water coefficient, Henry coefficient, function, behaviour and pharmacologic activity. PPCPs are largely consumed in modern societies that in many cases are indispensable for a comfortable and healthy life. Despite the fact that commonly grouped together, PPCPs are not the same and care should be taken when using this term. Similarly, the terms pharmaceuticals and Endocrine Disrupting Compounds (EDCs) are not synonymous.

Pharmaceuticals include prescription drugs, over the counter medicines and veterinary drugs. On the other hand, EDCs include a wide range of direct and indirect biological impacts and thus is difficult to have an available and definitive list of endocrine disruptor compounds.

Nowadays, more than 6 millions of PPCPs are commercialy available and their use is increasing 3-4% in weight every year (Kümmerer, 2003). Among them there are compounds belonging to a variety of therapeutic groups, such as: anti-inflammatories, tranquilizers, anti-depressives, antibiotics, contraceptives, antiepileptics, X-ray contrast media, etc. During and after consumption, human and animals excrete a combination of parent and metabolized pharmaceuticals which enter the environment through treated and untreated wastewater, usually reaching surface waters (Roberts and Thomas, 2006; Bester et al., 2008), groundwater (Drewes et al., 2003; Sacher et al., 2001), and occasionally even drinking water (Heberer et al., 2002; Ternes et al., 2002). Additionally, the compounds that comprise personal care products, such as skin care products, shampoo, soaps, sunscreen agents, washing lotions, etc. are consumed in enormous quantities. In the early 90s, annual production of these products exceeded 550 000 metric tons for Germany alone (Daughton and Ternes, 1999). Currently there are no legal requirements for the maximum concentrations of PPCPs in the environment.

1.2. SOURCE AND OCCURRENCE OF PPCPS IN WATER BODIES

PPCPs are continually released into the environment as a result of their manufacture (industrial sewage), use (via excretion) and improper disposal of unused or expired drugs which get directly into the domestic sewage system. Only a part of drugs is excreted in a metabolized form (more hydrophilic), whereas others drugs are excreted unaltered or in form of slightly transformed metabolites. Ingredients of personal care products enter wastewater via washing, bathing and showering and due to elevated lipophilicity of these compounds most of them are sorbed onto sludge and sediments. Agricultural practices are the higher source of antibiotics, which 40 percent is produced to promote the growth and fattening of livestock. In this sense, the manure normally used as fields fertilizer can contain traces of pharmaceutical compounds, which could be washed-out reaching local streams and rivers. Another relevant source of PPCPs is the effluents from hospitals. Furthermore, during sewage treatment, a significant fraction of these compounds will not be quantitatively removed, remaining in the

effluents and finally reaching surface waters and groundwaters (Mohle et al. 1999; Doll and Frimmel 2003).

During the treatment in STPs, a distribution between the dissolved and the solid fraction, including primary and secondary sludge, will take place. This partition is particularly relevant for the most lipophilic compounds. Therefore, the release of non-degraded PPCPs into the environment will occur with the final effluent of the plant, as well as with the excess sludge, which has been reported to contain pharmaceutical substances (Khan and Ongerth, 2002; Kupper et al., 2004; Kinney et al., 2006). If sewage sludge is disposed on agricultural lands, PPCPs pollution will not only reach surface water, but also groundwater and drinking water. Figure 1.1 shows the main sources and distributions of micropollutants in the environment.

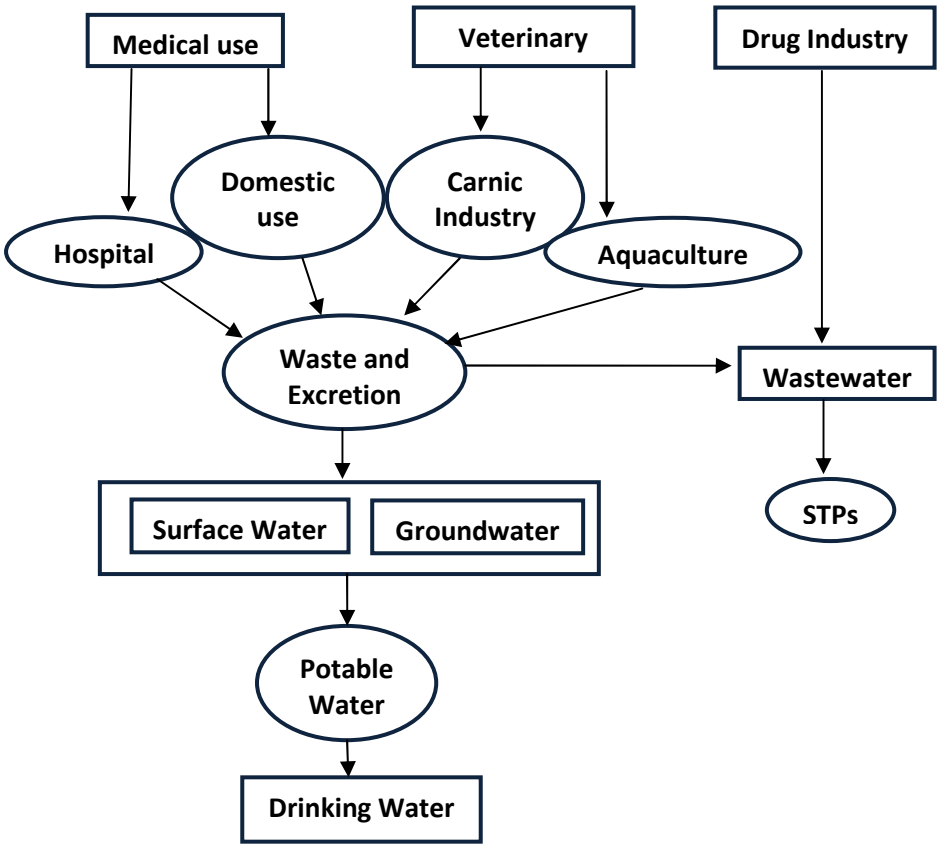

Figure 1.1. Sources and distribution of PPCPS in the environment (modified from Prados, 2010).

1.3. ENVIRONMENTAL EFFECTS AND RISKS

Micropollutants are denoted as "emerging" because information about their occurrence is fairly recent, as it is the case of PPCPs. However, these compounds have been discharged into the environment along decades, mainly in water bodies (Ternes and Joss, 2006). Despite the fact that these compounds are present at low concentrations, some ecotoxicological reports have shown that certain active compounds present in the environment, such as antidepressants, may cause premature reproduction in molluscs, while the presence of natural and synthetic hormones at extremely low concentrations (ng L^{-1}) can cause significant disruptions, such as fish feminization (Fent et al., 2006).

In recent years, researchers such as DeLorenzo and Fleming (2008) found new environmental effects of six PPCP compounds (simvastatin, clofibric acid, triclosan, fluoxetine, diclofenac, and carbamazepine) on the marine phytoplankton species *Dunaliella tertiolecta.* The results indicated that all PPCPs tested had a significant effect on the *D. tertiolecta* population. Flippin et al. (2007) observed that when Japanese medaka fish is exposed to the levels in the range of 1-100 μg L^{-1} of ibuprofen, their pattern of reproduction was altered. Also, some adverse effects (cellular toxicity and oestrogenic effects) were detected with diclofenac at 1 μg L^{-1} (Hong et al., 2007). Further studies of mixtures of certain compounds indicated stronger synergistic effects, compared with the effect that one of them could have alone or combined with another group of compounds. For example, it was found that a mixture of clofibric acid (lipid regulator) and fluoxetine (an antidepressant) causes morphological abnormalities in *Daphnia magna*. Furthermore, if the concentration of clofibric acid in the mixture increases, there is a significant increase of mortality. This indicates that proportion of each compound in the mixture influences the toxic effects. Furthermore, Cleuvers (2004) has evaluated the ecological potential of anti-inflammatory drugs and of diverse acting pharmaceuticals in different sets of biotests using different aquatic organisms. A mixture of Nonsteroidal Anti-Inflammatory Drugs (NSAID) diclofenac, ibuprofen, naproxen, acetylsalicylic acid has been evaluated using acute *Daphnia* and algal tests. Toxicity of the mixture was found at concentrations at which the single compound showed no or only little effects.

Regarding risk assessment, endocrine impacts on marine life have already been identified. Mainly emphasis should also be put on compounds that can

bio-accumulate in the aquatic biota (Brausch and Rand, 2011). This is the case of the fragrances galaxolide and tonalide, where concentrations measured in fish were 600 times higher than the nominal test doses applied (Schreurs et al., 2004). In addition, the main concern about the release of antibiotics into natural waters is the eventual formation of microbial resistance to these antibiotics, meaning that an increasing number of infections could no longer be treated with the current medicines (Kümmerer, 2009).

1.4. HISTORICAL DEVELOPMENT OF ACTIVATED SLUDGE TREATMENT PLANTS

Over the course of time, several processes have become integrated in the activated sludge system in order to cover a wide range of contaminants removal (Figure 1.2). During the first half of the 20th century, the first treatment plants were simple and their consisted basically of primary treatment, i.e. screens, sieves and settling tanks. These systems were designed to operate with a Sludge Retention Time (SRT) less than 5 days and with the only purpose to remove easily biodegradable organic matter. At the end of 60s, the focus of treatment plants was to remove phosphate using chemical precipitation and in the 70s, nitrogen originated mostly from urine was the goal of treatment plants. Moreover, in these decades the systems increased their SRT up to 8-12 days. In the 80s, nitrification was supplemented with a partial denitrification in which the nitrate is converted to molecular nitrogen and in the 90s an anaerobic zone was introduced to enhance biological phosphate removal. Moreover, the systems in the last two decades operated with a slight increase of SRT of around 14-20 days.

Through the advance in analytical techniques, PPCPs have increasingly been detected in water bodies, and thus represent a new possible challenge for the Sludge Treatment Plants (STPs). Moreover, the current STPs widely used today do not remove these micropollutants to an acceptable extent (Johnson and Sumpter 2001; Kolpin et al. 2002; Williams et al. 2003; Schultz and Furlong 2008). Therefore, physical, chemical and biological methods for micropollutants removal are currently being evaluated and developed to upgrade the existing treatment systems and enhance the removal of organic micropollutants.

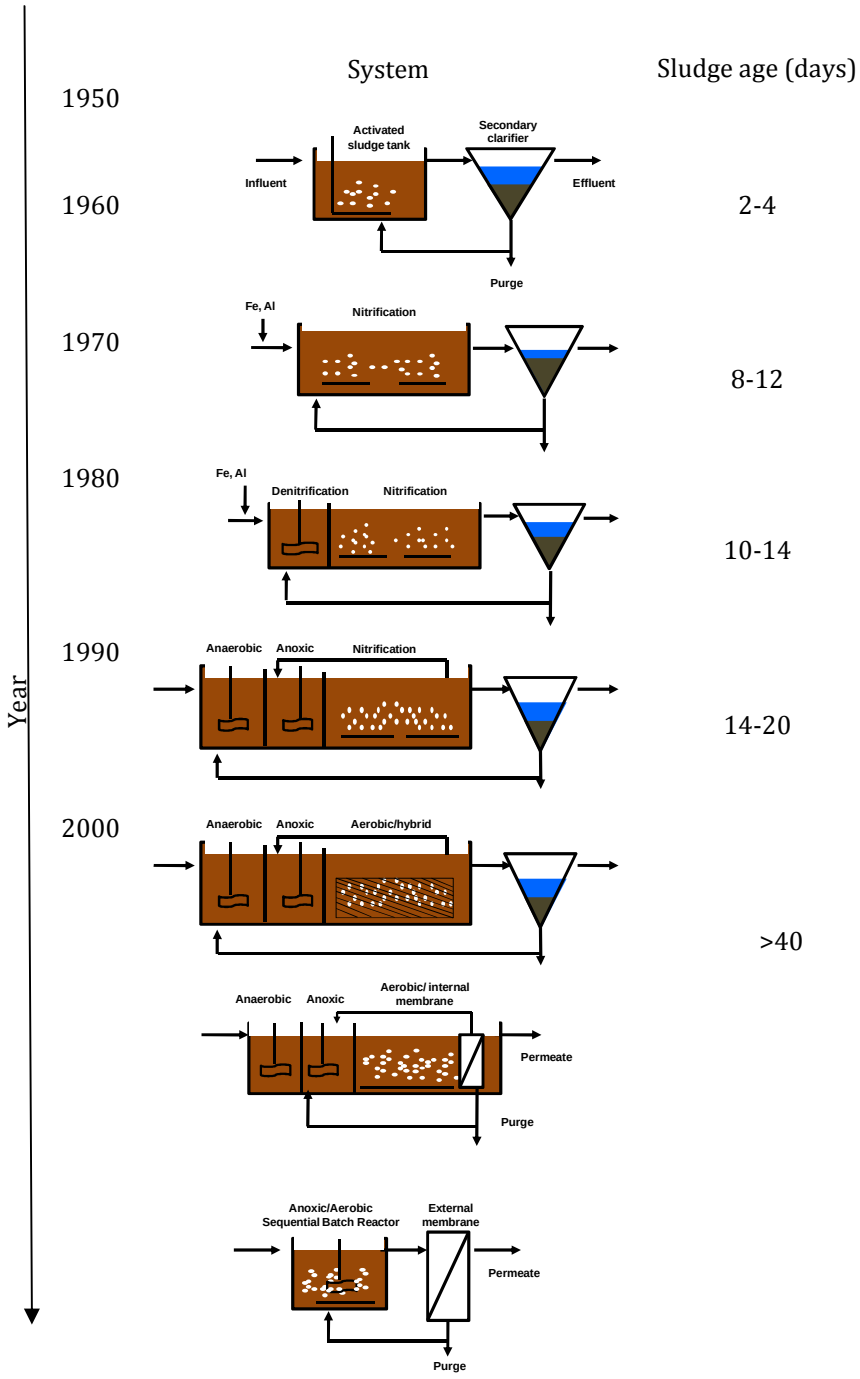

Figure 1.2. Historical development of activated sludge treatment plants (Modified from Ternes et al., 2004).

1.5. REMOVAL OF PPCPS IN STPs

The current sewage treatment plants have been not designed to deal with the presence of PPCPs due to their low concentrations (μg L^{-1} for pharmaceuticals or ng L^{-1} for hormones) in comparison with the compounds (organic matter and nutrients) that are commonly removed (Figure 1.3). Moreover, PPCPs removal efficiencies varied widely among plants and may depend of the configuration and operating mode of the plant.

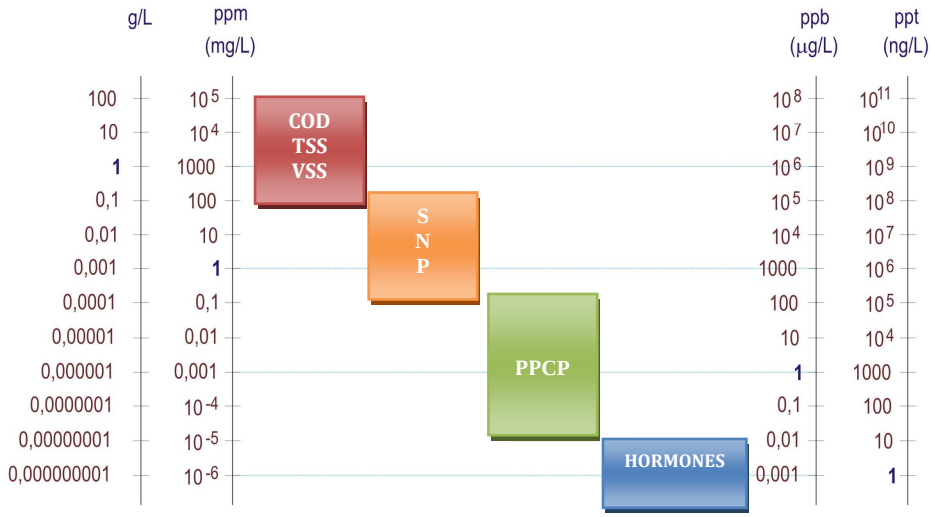

Figure 1.3. Different range of pollutants concentration presented in wastewater.

According to the results obtained especially during the 90s and early 2000s, there are very significant differences in micropollutant removal efficiencies reported from sewage treatment plants, which indicate that we are facing a complex group of substances with different physic-chemical properties, different removal mechanisms involved, etc. (Carballa et al., 2004; Joss et al., 2006; Zorita et al., 2009). An example of substance with high variability is diclofenac, for which removal efficiencies between 17% (Heberer, 2002), 69% (Ternes, 1998) and 100% (Thomas and Foster, 2004) have been reported. On the other hand, the same behaviour has been found for the antiepileptic carbamazepine in different STPs, with removal efficiencies <10% thus showing recalcitrant behaviour (Ternes, 1998; Heberer, 2002; Clara et al., 2004). Therefore, more work is needed in order to elucidate the

main factors involved in PPCPs removal for each substance under different process configurations.

Once a micropollutant is discharged into wastewater, it will be distributed between the different environmental compartments (e.g. surface water, soil, sediment) according to their physico-chemical properties. Furthermore, its persistence will depend on its resistance to biological or abiotical degradation. Therefore, the most important PPCPs removal mechanisms within STPs are: i) sorption to suspended solids in the wastewater, which are removed by sedimentation as primary and secondary sludge in the primary and secondary clarifiers; ii) decomposition of compounds through bacteria in the activated sludge, which is designated as biological transformation or mineralization; iii) abiotic degradation and stripping by aeration is taking into account only for some substances.

1.5.1. Removal mechanisms of PPCPs in STPs

1.5.1.1. Sorption

In the case of sorption of organic micropollutants, a distinction is made between two main mechanisms (Ternes et al., 2004):

- Absorption: Processes in which molecules present in a given fluid enter into another bulk phase. It refers to hydrophobic interactions of the aliphatic and aromatic groups of compounds with the lipophilic cell membrane of some microorganisms and the fat fractions of the sludge.
- Adsorption: It is an electrostatic interaction of positively charged groups (e.g., amino groups) with the negatively charged surfaces of the microorganisms.

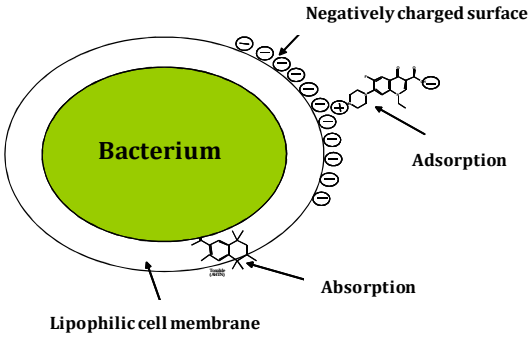

Figure 1.4. Important mechanisms carried out during the sorption of organic micropollutants.

The quantity of a substance sorbed per litre of wastewater C_{sorbed} (g L^{-1}), is expressed as a simplified linear equation (Siegrist et al., 2004):

$$C_{sorbed} = K_d \cdot SS \cdot C_{dissolved} \quad (1)$$

where K_d is the sorption constant (L g^{-1}), defined as the partition of a compound between the sludge and the water phase; SS (g L^{-1}) represents the concentration of suspended solids in the activated sludge tank; and $C_{dissolved}$ (g L^{-1}) is the dissolved concentration of the substance. Moreover, the sorption potential of PPCPs is a function of both their lipophilic character (K_{ow}) and acidity (pKa).

Sewage sludge is an important indicator for documenting the anthropogenic loading of problematic substances through the waste water. It is therefore important to retain the quality control before the use of sewage sludge in agriculture.

1.5.1.2. Biological transformation

Biodegradation defines the reaction processes mediated by microbial activity (biotic reaction). In the case of the biological degradation of trace substances, a distinction is made between:

- Co-metabolism, in which the bacteria only partly break down or convert the trace substances and do not use it as a carbon source.
- Biodegradation, in which the bacteria use the trace substance as a carbon and energy source and hence totally mineralize it.

In aerobic processes, microorganisms can transform organic molecules via the succession of oxidation reactions to simpler products for instance other organic molecules or a complete mineralization to CO_2 (Siegrist et al., 2004; Van der Meer et al., 2006). The decomposition also increases with the age of the sludge; the reason is that the bacterial symbiosis becomes more diversified because slower growing bacteria can also grow in the sludge. At low concentrations, the kinetics of decomposition of micropollutants occurs mostly according to the first order reaction (Siegrist et al., 2004):

$$R_{biol} = k_{biol} \cdot SS \cdot C_{micropollutant} \quad (2)$$

where R_{biol} (mg L^{-1} d^{-1}) is the biological degradation rate, k_{biol} is the biological degradation constant, SS (g L^{-1}) is the concentration of suspended solids and $C_{micropollutants}$ (mg L^{-1}) is the concentration of micropollutants in influent supposed to be degraded. According to these degradation constant values,

Joss et al. (2006) differentiated three groups of compounds: i) no removal, with k_{biol} <0.1 L kg^{-1}SS d^{-1}; ii) partial removal with 0.1< k_{biol} >10 L kg^{-1}SS d^{-1}; and iii) transformation by more than 90% with k_{biol} >10 L kg^{-1}SS d^{-1}. In addition, the degradation rates are strongly dependent upon environmental conditions, such as the redox potential of the systems and the microbial populations present.

1.5.1.3. Abiotic degradation and stripping

Abiotic degradation comprises the degradation of organic chemicals via chemical (e.g., hydrolysis, oxidation) or physical (e.g., photolysis) reactions (Doll and Frimmel 2003; Bouillon and Miller 2005; Iesce et al., 2006). Abiotic processes are not mediated by bacteria and have been found to be of quite limited importance in wastewater compared to the biodegradation of micropollutants (Katsoyinnis and Samara 2005; Soares et al., 2006). The removal of micropollutants by stripping during the activated sludge process depends on vapour pressure (Henry coefficient) and octanol-water partition coefficient (K_{ow}) of the analysed micropollutant, and becomes significant when the Henry's law constant (H) ranges from 10^{-2} to 10^{-3} (Stenstrom et al., 1989). At very low H/K_{ow} ratio, the compound tends to be retained by particles (Galassi et al., 1997). The rate of volatilization is also affected by gas flow rate and therefore, high efficiency submerged aeration systems such as fine bubble diffusers should be used to minimize volatilization rates in wastewater treatment plants (Stenstrom et al., 1989).

1.5.2. Factors affecting removal of PPCPs

Mechanisms of removal do not follow a general rule since their relative contribution depends on the physico-chemical properties of the micropollutant, the origin and composition of the wastewater, and the operational parameters of the wastewater treatment facility. The following main factors could affect the removal of organic micropollutants during STPs.

1.5.2.1. Temperature

Seasonal variations in removal of some micropollutants in full scale STPs were reported in several studies when comparing the total loads of compounds in the influent and effluent (Vieno et al. 2005, Castiglioni et al. 2006), in a way that higher removals were measured at higher temperatures. It is known that temperature can affect sorption and biodegradation rates of micropollutants during sewage treatment. For most compounds, equilibrium sorption decreases with increasing temperature (Hulscher and Cornelissen

1996), while microbial activity is enhanced at higher temperatures. Suárez (2008) reported the effect of temperature on removal of some pharmaceuticals in a denitrifying/nitrifying pilot plant. This studied showed that the effect of the temperature is dependent on the magnitude of the pseudo first-order degradation constant of the considered compounds and it is mainly noticeable for micropollutants with moderate k_{biol} such as erythromycin and sulfamethoxazole.

1.5.2.2. Microbial diversity, adaptation and co-metabolism

Biodegradation of pollutants can in principle either be achieved by metabolism, using the pollutant as a source of primary carbon or nutrients for growth and/or as energy source; or by co-metabolism, in which the pollutant is transformed by the action of extracellular enzymes produced by the cells, but without any benefit for the microorganism. There is little information so far concerning this specific issue in the case of micropollutants, although due to the very low concentrations of such compounds it seems more plausible that bacteria grow on a primary substrate. In this way, Forrez et al. (2008) linked the enzyme ammonium monooxygenase, involved in nitrification processes, with the degradation of xenobiotic substances such as EE2. Drillia et al. (2005) found that the antibiotic sulfomethoxazole was used as carbon and nitrogen source by an enriched consortium. However, it was degraded whenever there was a depletion of carbon and nitrogen or both in the medium, while in the presence of acetate and ammonium nitrogen (alternative carbon and nitrogen sources, respectively), sulfomethoxazole was not degraded and remained intact.

The possibility that existing microorganisms in biological processes can acclimate to the presence of xenobiotic compounds by broadening their enzymatic spectrum was pointed out by several authors under different operational conditions. Suárez (2008) reported possible acclimation phenomena in a denitrifying/nitrifying pilot plant, which after achieve stable conditions (ensured a diversified biota including slowly-growing bacteria) was feed with target compounds. Concerning naproxen a gradual increase in its removal from 27% up to 99% was observed during the first 300 days. Taking into account that removal of naproxen remained stable during the following 300 days, this initial enhancement was attributed to a possible acclimation of bacteria to this pharmaceutical.

1.5.2.3. Biomass concentration and structure

In relation to SRT, the biomass concentration is very important. Sorption of micropollutants is favoured by the high biomass content, which is particularly characteristic for MBR (Cirja et al., 2008). Different researches showed the effect of biomass concentration on micropollutants removal. For example, Kim et al. (2005) carried out adsorption kinetics for tetracyclines at different biomass concentrations in sequencing batch reactors. Results showed that the lower degradation rates were found to the reduced biomass concentration. Suarez et al. (2008) observed an increase in the removal efficiency of diclofenac to maximum levels of around 75% after a growing of nitrifying bacteria in a denitrifying/nitrifying pilot plant.

The structure of biomass is expected to have an impact on the removal of micropollutants, as it affects mass transfer between the target compound and microorganisms (i.e. the availability of the compound), the viability of bacteria and their enzymatic activity, although this aspect should be further studied. Several operational parameters, such as SRT, composition of the treated wastewater, selection of technology, etc., may have an impact on the structure of the biomass developed in the system. For example, Cicek et al. (1999) reported different biomass structures depending on the technology used for wastewater treatment, indicating that MBR sludge was composed of smaller flocs and contained many free-living bacteria, while the AS system was composed of larger flocs and comparatively higher amounts of filamentous organisms.

1.5.2.4. Hydraulic Retention Time (HRT)

Following IUPAC terminology Hydraulic Retention Time (HRT) is the average time a particle resides in a bioreactor through which a liquid medium continuously flows. The biological degradation kinetics will be the key point in establishing if this parameter does or not affect the efficiency of the process. Huang et al. (2008) studied the degradation of plasticizer di-(2-ethylhexyl)phthalate (DEHP) in an anaerobic-anoxic-aerobic activated sludge reactor, in which the overall removal efficiency of the compound was independent of the considered HRT, although the contribution of the individual processes to that removal was influenced by the HRT. The study revealed that anaerobic degradation of DEHP increased from 14% to 23% when the HRT of the reactor was incremented from 5 h to 14 h, indicating that the process with the slowest kinetics was positively influenced by higher HRT. Similarly, Tauxe-Wuersch et al. (2005) determine the influence of HRT

on the removal of acidic drugs in full-scale STPs with primary and secondary treatment showing that their behaviour depend on the physico-chemical properties of e specific compound. On the other hand, Metcalfe et al. (2003) reported high persistence of some compounds such as CBZ which are independent of the HRT.

1.5.2.5. Sludge Retention Time (SRT)

Sludge retention time (SRT) is the mean residence time of microorganisms in a system. Many studies state that sufficient high SRT is essential for the removal and degradation of micropollutants from wastewater and make possible the enrichment of slowly growing bacteria and also the establishment of a more diverse biocoenosis able to degrade a large number of micropollutants. It was demonstrated that at short SRTs (<8 d) those bacteria are removed from the system and in this case, the biodegradation is less significant and adsorption to sludge will be more important (Jacobsen et al. 1993). A diversified microbiocoenosis can develop at SRT higher than 8 d, including also nitrifying bacteria.

By varying the SRT in MBRs, Lesjean et al. (2005) noticed that the removal of pharmaceuticals residues increased with a high sludge age of 26 d and inversely decreased at a lower SRT of 8 d. Kreuzinger et al. (2004) reported the elimination of Diazepam at STPs with sludge retention times ranging from 0.7 to 275 days. In all cases the removal efficiency did not surpass 25% with no removal for the SRT equal to 0.7 days. Clara et al. (2005) reported almost complete removal of ibuprofen, ketoprofen and indomethacin only at SRT >20 d.

1.5.2.6. Use of additives

Some modifications, such as the use of chemical additives, can be implemented in STPs in order to improve solid and fat separation and, consequently, to enhance the removal of those substances with high sorption properties.

Coagulation-flocculation has been shown to be a suitable pretreatment option for decreasing at some extent the amount of certain type of micropollutants in urban or hospital wastewaters. Carballa et al. (2005) showed that the use of ferric and aluminium salts enhances the removal of substances with high sorption properties, such as musk fragrances, up to 50-70%. Furthermore, the presence of trivalent cations could enhance the elimination of acidic compounds (such as naproxen) by ionic or quelating

interactions. Similar conclusions were obtained by Suárez et al. (2009) working with hospital wastewaters, who found high removal efficiencies for fragrances such as galaxolide (>90%) which was attributed to their strong lipophilic character.

Powdered or granular activated carbon (PAC and GAC) have been shown to effectively enhance sorption of organic micropollutants like pesticides or taste and odour compounds (Newcombe et al. 1997). The most common applications of these adsorbents are as a final step in drinking water plants or as postreatment in STP effluents. Westerhof et al. (2005) using a bench-scale simulation water treatment plant model and natural waters spiked with 62 different EDCs/PPCPs provided the results which are summarized in Table 1.1. This study showed no significant removal except using activated carbon (powder) and oxidation via chlorination and ozonation. Chemical treatment such as coagulation, flocculation or lime softening shows ineffective removal for EDCs and PPCPs.

Table 1.1. Removal performance of EDCs/PPCPs in selected drinking water process (Bolong et al., 2009).

Treatment process	Removal performance
Coagulation by alum or ferric sulphate	<20% of compound removed, specially associated with particulate matter.
Lime softening	<20% of compound concentration was removed at pH 9 - 11
Powdered Activated Carbon (PAC)	>90% of many EDCs removed (at 5 mg/L dose PAC of 4 hour contact time). However, some EDCs (ibuprofen, sulfamethoxazole, meprobamate) had lower removals (40–60%). Hydrophobic compounds (log Kow >5) have better removal than polar compounds.
Biofilm	Removal depends on biodegradability of compounds but removal rate is unclear.
Chlorination	Able to remove >90% for more reactive compounds containing aromatic structures with hydroxide functional groups. Not suitable because it produces chlorine by-product (react with EDCs) and should be avoided.
Ozonation	Oxidized similar to chlorination but at slightly higher removal rates. Addition of hydrogen peroxide during ozone addition slightly increased the EDCs removal

1.6. TREATMENT TECHNOLOGIES

Conventional activated sludge (CAS) processes have been widespread used for wastewater treatment, and so far it is known that these processes are only partially effective in the removal of organic micropollutants. Therefore, new treatment strategies have been proposed by the reformulation of CAS systems by including different innovative steps such as UF or MF membranes, the use of adsorbents, the use of Advanced Oxidation Processes (AOPs), etc.

MBR technology, which is based in the combination of a suspended biomass biological reactor and a membrane microfiltration or ultrafiltration step, has gained wider acceptance throughout the industry. One of its major advantages is the potential production of a high quality effluent which can be even appropriate for water reclamation. Moreover the results reported so far concerning micropollutants are not significantly clear enough to show its advantages with respect to AS systems. In fact, both technologies, MBRs and CAS systems, have been shown to achieve comparable results in terms of micropollutants removal when operating at similar conditions (Weiss and Reemtsma, 2008).

In this way, the potential of the use of additives such as activated carbon either in its granular o powdered form combined with the advantages of MBRs (higher SRT and sludge concentrations, complete retention of biomass and PAC) could be a good option in order to enhance the removal of the target compounds.

1.7. ACTIVATED CARBON

Although adsorbents have been used during decades in wastewater treatment, there is a certain trend in order to consider them again in the configuration of new innovative biological treatment plants such as MBR processes, hybrid systems, etc. (Tewari et al., 2006; Gupta et al., 2009; Li et al., 2010). Table 1.2 shows a summary of the main types of adsorbents used for wastewater treatment.

Table 1.2. Main types of adsorbents used for wastewater treatment.

Adsorbent	Form	Surface area $m^2 g^{-1}$	Examples of uses	Reference
Alumina	Granules of different sizes	200-300	Used in packed beds to remove contaminants such as fluoride, arsenic, selenium, silica, and natural organic matter	Ghorai and Pant , 2005
Silica gel	Porous and non-crystalline, granules with different sizes	250-900	Used in industries for drying of gases and liquids, also for dyes adsorption	Do, 1998
Zeolite	Crystalline	350-700	Ion-exchanged beds for removal of specific organic compounds	Sousa-Aguilar et al., 1998
Activated carbon	Granules powder, cylindrical, pellets and spherical	500-2000	Due to versatility of activated carbon, it can be applied for the treatment of industrial wastewater effluents, municipal wastewater, landfill leachate, etc.	Días et al., 2007.

Activated carbon (AC) is well known as very effective adsorbent due to its high porosity, large surface area (than can reach 3000 $m^2 g^{-1}$), variable characteristics of surface chemistry and interactions with adsorbate/adsorbent (Días et al., 2007; Lei et al., 2002). The interactions can be electrostatic (if the adsorbate is an electrolyte that dissociates in aqueous solution) or non-electrostatic. Moreover, the nature of the electrostatic interactions can be attractive or repulsive, depending on the: i) charge density of the carbon surface; ii) chemical characteristics of the adsorbate; and iii) ionic strength of the solution. Non-electrostatic interactions are always attractive and can include: i) van der Waals forces; ii) hydrophobic interactions; and iii) hydrogen bonding (Días et al., 2007).

According to Moreno-Castilla (2004), some of characteristics of adsorbate that mainly influence adsorption processes with activated carbon are: the molecular size, solubility, dissociation constant (pKa) and the nature of the substituent (in the case of aromatic adsorbates). The molecular size establishes the accessibility of the adsorbate to pores of the carbon, the solubility determines the degree of hydrophobic interactions between the adsorbate and carbon surface and the pKa controls the dissociation of the adsorbate.

Activated carbon used for water treatment is available in two main forms. If small particles compose the raw material for activated carbon preparation, as in the case of wood sawdust, **powdered activated carbon (PAC)** is obtained. In contrast, if the raw material is hard enough to be milled into relatively large and hard particles, as in the case of coconut shells, **granular activated carbon (GAC)** can be obtained (Tancredi et al., 2004). The main difference between GAC and PAC is the particle size, PAC available commercially having between 65 and 90% of the particles passing through a 325 mesh (±45 μm) sieve (water quality and treatment, 1990).

GAC is commonly used as a filter for gas or liquid applications and is regenerated after use. Typical systems consist of beds, columns or cartridges traversed by liquid or gas (Figure 1.5). On the other hand, PAC is applied during the conventional treatment mixed with the sludge either in Conventional Activated Sludge (CAS) systems (Figure 1.5) or a Membrane Bioreactor (MBR). GAC column effectiveness is a function of the water loading rate and empty bed time, whereas PAC effectiveness is also a function of the carbon dosage. Table 1.3 shows the main advantages and limitations of using either PAC or GAC in water treatment.

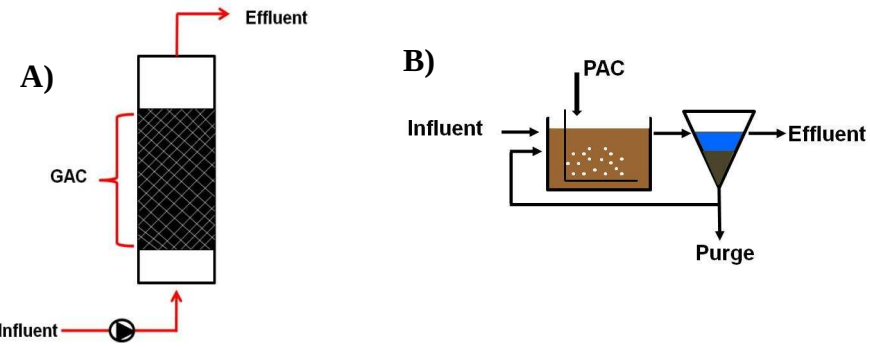

Figure 1.5. Use of activated carbon: A) GAC in adsorption column and B) PAC in the aeration tank of CAS system.

Table 1.3. Advantages and limitations of using either GAC or PAC form in wastewater treatment (Freese et al., 2000; Tancredi et al., 2004; Yu, 2007).

Filter media	Advantages	Limitations
Granular Activated Carbon (GAC)	• Long contact time • Easy separation and regeneration • Lower carbon usage rates • Low residual concentration levels	• Must be regenerated when adsorption capacity is depleted • High initial and maintenance costs
Powdered Activated Carbon (PAC)	• Low capital cost • Dosages can be adjusted easily in response to influent concentration changes • Improved process stability • Increased SRT • Improved sludge settleability • Reduced effluent toxicity	• High operating cost (if high dosage required for long period of time) • Inability to regenerate • Difficult for sludge disposal

1.7.1 Advanced treatment with activated carbon

Since the application of conventional biological treatments was shown to be not effective to achieve a complete removal of PPCPs present in wastewater, new strategies are being considered to enhance the removal of these trace substances. The addition of AC directly to the aeration tank has been considered an attractive option (Babel and Kurniawan, 2003; Derbyshire et al., 2001; Gupta et al., 2006), since some studies confirmed that the presence of AC in activated sludge systems can effectively increase the removal efficiency of conventional pollutants and even some persistent compounds, by the combination of both adsorption and biodegradation. In this way, Xiaojlan et al. (1991) studied the mechanisms involved in a Biological Activated Carbon (BAC) test using an adsorption column with phenol as a testing pollutant. A simple combination of biodegradation and adsorption were considered the main mechanisms involved in the BAC process according to different assays, such as AC adsorption capacity, respirometric assays and comparative test between BAC and aerated filter, which were carried out. Moreover, Sirotkin et al. (2001) reported a synergetic combination of biodegradation and carbon adsorption of non-ionogenic surfactants (products of the organic synthesis of polyoxyethylen) during

wastewater treatment in a BAC process, which can lead to the biological regeneration of the adsorbent as well as to a lower toxicity caused by these substances. In addition, Aktas and Cecen (2007) carried out different tests using different activated carbon types with 2-clorophenol as a testing pollutant, showing the effect of combined adsorption and biodegradation and a easier biodegradation of this compound in the presence of AC.

Powdered Activated Carbon (PAC) and Granular Activated Carbon (GAC) are commonly used for sorption of organic micropollutants like pesticides, taste and odour (Ternes and Joss, 2006), heavy metals (Khezami and Capart, 2005) or dyes (Crini, 2006). Moreover, some researchers (Thuy and Visvanathan, 2006; Aktas and Cecen, 2001) reported that the presence of AC is a suitable support for bacterial attachment, enabling and enhancing the retention of the slower growing bacteria, such as nitrifiers. On the other hand, it is commonly considered that the adsorbability of micropollutants is significantly lower when they are in competition with background organic matter, as would be the case of direct activated carbon additions into biological aeration tanks (Snyder et al. 2007).

Aktas and Cecen (2007) summarized the advantages of PAC addition to the activated sludge treatment processes such as:

- improvement of the sludge settling.
- improvement of the thickening/dewatering of the sludge
- increase of organic, refractory and inhibitory compounds removal
- reduction of the impact of organic shock loadings
- suppression of aerator foaming and bulking
- nitrification improvement
- increase the SRT

Furthermore, there are studies that show different advantages using AC, either in powdered or granular form, in activated sludge system (Table 1.4a, b).

Additionally, Membrane Bioreactors (MBRs) have been widely recognized due to the ability to produce higher quality effluents in terms of conventional pollutants, low sludge production, small foot print and flexibility in the case of future expansion (Li et al., 2010). However, it is not clear if these systems may effectively enhance the removal of organic micropollutants since similar results have been obtained between CAS and MBR systems. Since it is considered that adsorption of micropollutants onto sludge is the first step in

order to accomplish their removal in biological processes, the addition of adsorbents such as powdered or granular activated carbon directly into the biological reactors could lead to a higher retention of soluble micropollutants (which did not interact with sludges) into the system, which could influence biodegradation. In many cases these adsorbents have been used to decrease the liquid concentration of toxic substances (Papadimitriou et al., 2009; Lesage et al., 2008). Tables 1.5a, b summarise different works carried out with the addition of PAC into the aeration tank of MBR systems.

Table 1.4a. Addition of either PAC or GAC in different conventional activated sludge systems.

Type of water treated	Reactor characteristics	Adsorbent and doses	Main improvements due to PAC addition	References
Mixture of landfill leachate and domestic wastewater	Batch activated sludge reactor	PAC (NORIT SA): 0.1-3.5 g L^{-1}	• Improvement of COD removal >90% • Enhanced nitrification from 0% to 30%	Aktas and Cecen, 2001
Synthetic wastewater (containing phenol)	Laboratory scale activated sludge reactor (3.5 L)	PAC (sewage sludge and Chemviron): 1 g L^{-1}	• Enhanced phenol removal from 58 to 99% and COD removal from 87 to 93% • Increased stability and enhanced the performance of the CAS process	Martin et al., 2004
Synthetic solution with a mixture of phenol and *p*-methylphenol	Sequencing Batch Reactor (SBR) operating with cycles of 12 h	PAC: 4 g L^{-1}	• Reduction of phenol to 90% by adsorption • COD removal increased to around 40% • Enhanced bioactivity of biomass	Lee and Lim, 2005
Synthetic wastewater (medium strength) with Cu (II) and Cd (II)	Sequencing Batch Reactor (SBR): 10 L	PAC: 1 g L^{-1} (cycles of 6 h)	• Improved COD removal efficiency • Increased the oxygen uptake rate of activated sludge microorganisms by reducing the toxic effect of metal • Cu^{2+} and Cd^{+2} removal ratio was around 80% and 70%, respectively	Ong et al., 2005

Table 1.4b. Addition of either PAC or GAC in different conventional activated sludge systems.

Type of water treated	Reactor characteristics	Adsorbent and doses	Main improvements due to PAC addition	References
Landfill Leachate	Laboratory scale activated sludge reactor (16 L)	PAC: 3 g L^{-1}	• Enhanced the overall performance of the system • Enhanced COD removal from 8 to 20% • Enhanced $N\text{-}NH_4^+$ from 45 to 60% • Enhanced colour removal from 1 to 50%	Aghamohammadi et al., 2007
Synthetic textile wastewater	Sequencing Batch Reactor (SBR) with 7,5 L	GAC (CGC-11): 1 g L^{-1}	• Dye removal efficiency increased by around 6-8% • Total bio-sludge 10% higher than without GAC addition • Lower SS in the effluent after GAC addition • Lower SVI (<100 mL g^{-1}) • Higher SRT • Less sludge production GAC as a media for biofilm to attach to	Sirianuntapiboon and Srisornsak, 2007
Coke oven synthetic wastewater	Sequencing Batch Reactor (SBR): 5 L and conventional system (12 L)	PAC (SAE 2, NORIT): 1 g L^{-1}	• Improvement of organic removal as well as decrease of toxicity (30-40%) caused by phenol and cyanides • Better efficiency of both system after PAC addition, enabling the production of effluents with low toxicity, imposing minimal environmental risks.	Papadimitriou et al., 2009

Table 1.5a. Addition of PAC into the aeration tank of MBRs.

Type of water treated	Reactor characteristics	Adsorbent and doses	Main improvements due to PAC addition	References
Synthetic municipal wastewater (high strength)	Laboratory-scale MBR (14 L) using submerged PVDF hollow	PAC (PS1000): 1 g L^{-1}	• Enhanced carbamazepine removal from 12% to 92% • Enhanced sulfamethoxazole removal from 64% to 82%.	Li et al., 2010
Municipal wastewater	Pilot-scale MBR (85 L) using 5 homemade PVPF flat sheet membrane	PAC (NORIT SAE): 0.5 g L^{-1}	• Improvement of the critical flux of around 10% • Increase of the filtration period without significant fouling at high fluxes 50-72 L m^{-2} h^{-1}. • Decrease of gel deposition on membrane surface • Slight increase of the permeate quality • Easier removability of gel deposition at high flux • Slight increase of the permeate quality.	Remy et al., 2009
Wastewater from an alcohol distillery (high strength)	Laboratory-scale MBR (8 L) using submerged nylon mesh	PAC (wood material): 2 g L^{-1}	• SVI was decreased • Sludge dewaterability was improved • EPS composition was altered (proteins/carbohydrate ratio).	Satyawali and Balakrishna, 2009

Table 1.5b. Addition of PAC into the aeration tank of Membrane Bioreactors (MBRs).

Type of water treated	Reactor characteristics	Adsorbent and doses	Main improvements due to PAC addition	References
Synthetic industrial wastewater and 2,4-dimethyl-phenol (DMP)	Laboratory-scale MBR using submerged hollow fiber membrane	PAC (coconut material): 1 g L^{-1}	• Slight decrease of the sludge production • Reduction of fouling rate and the modification of cake deposit and biofloc structure, • Increase of the resistance to toxic substances due to a rapid adsorption of this molecule on adsorbent (>99%) followed by biodegradation (in the case of DMP).	Lesage et al., 2008
Synthetic municipal wastewater (medium strength)	Laboratory-scale MBR using polyethylenen hollow fiber membrane	PAC (wood material): 5 g L^{-1}	• High effluent quality • Reduce membrane fouling and therefore decrease the transmembrane pressure.	Guo et al., 2008
Tannery industrial wastewater	MBR pilot plant using two hollow fiber module	PAC (Lambsorb): 1.5- 3 g L^{-1}	• High effluent quality (improvement in COD removal) • Better control of operation criticisms related to the fouling rate, fouling reversibility and membrane life cycle.	Munz et al., 2007
Synthetic municipal wastewater	Laboratory-scale MBR (17 L) using submerged hollow fiber membrane	PAC: 1.2 g L^{-1}	• Effective control flux reduction • Enhanced membrane permeability • Decrease of membrane fouling • Reduction of fine colloids and EPS.	Li et al., 2005

1.8. SELECTION OF PPCPS

In this study some representative compounds belonging to the main groups of PPCPs micropollutants was selected according to the following criteria: i) wide occurrence in sewage reported in different areas of the world; ii) available analytical methodology; iii) high consumption; and iv) research dataalready available. The complete list included pharmaceuticals from five different therapeutic classes (antibiotics, anti-depressants, anti-inflammatory drugs, tranquilizers and anti-epileptics), a synthetic hormone used in contraceptive drugs and cosmetic ingredients.

1.8.1. Endocrine-Disrupting Compounds (EDCs).

EDCs can be defined after Kavloc et al. (1996) and the US-EPA (United States Environmental Protection Agency) as an "exogenous agent that interferes with the synthesis, secretion, transport, binding action or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, development and or behaviour."

This group comprises a diverse range of both natural and synthetic chemicals; in this study the contraceptive 17α-ethinylestradiol (EE2) was used as representative EDCs found in environmental water compartments in low concentrations in the range of ng L^{-1}. It was reported that these substances may alter endocrine disrupting function at a certain extent.

1.8.2. Antibiotics

Antibiotics are considered as ‘‘pseudopersistent’’ contaminants due to their continual introduction into the ecosystem (Hernando et al., 2006). The occurrences of antibiotics in the environment and their potential adverse effects on organisms, such as resistance among bacteria pathogens, have therefore received considerable attention (Gulkowska et al., 2008; Kemper, 2008). For example, a study carried out by Costanzo et al. (2005) showed that a bacteria isolated from sewage bioreactors exhibited resistance to some antibiotics including trimethoprim, erythromycin, tetracycline, ciprofloxacin and ampicillin. Moreover, previous studies showed that most of the antibiotics exhibited direct toxic effects (Park and choi, 2008) and also, adverse effects in a mixture (Pomati et al., 2006) despite relatively low environmental concentrations of individual compounds.

It has been demonstrated that antibiotics are, in general, poorly absorbed by the human body, and thus are excreted either unchanged or transformed, via urine and faeces (McArdell et al., 2003). As a consequence, antibiotics can transport in the environment leading negative effects on water organisms (Chang et al., 2010), this work was focused on the study of three antibiotics: trimethoprim, erythromycin and roxitromicine.

1.8.3. Anti-inflammatories Drugs

The Nonsteroidal Anti-Inflammatories Drugs (NSAIDs) ibuprofen, naproxen and diclofenac are organic micropollutants with pKa values ranging from 4.0 to 5.7 and thus, belong to the group of acid pharmaceuticals. NSAIDs represent one of the most important groups of pharmaceuticals in the world with an estimated annual production of several kilotons (Cleuvers, 2004). During the last years many studies have shown that NSAIDs are present in measurable concentrations in the aquatic environment. For example, Stumpf et al. (1996) were the first to identify a group of anti-inflammatories such as diclofenac, ibuprofen, acetylsalicylic acid and ketoprofen in sewage and river waters. Moreover, Ternes (1998) reported levels up to 1 μg L^{-1} of diclofenac, idometacine, ibuprofen, naproxen, ketoprofen and phenazone in STPs effluents.

Furthermore, some acute toxicity studies of NSAIDs have been reported, such as researches carried out by Cleuvers (2004) that evaluated the ecotoxicity of diclofenac, ibuprofen, naproxen and acetylsalicylic acid using *Daphnia* and algal test. The results showed that the toxicity of the mixture was significant, even at concentrations at which the single substances showed no or only very slight effects. As a consequence of the possible effects on aquatic organisms by the presence of NSAIDs in the environment, this project was focused on the study of diclofenac, ibuprofen and naproxen as representative compounds of anti-inflammatory drugs.

1.8.4. Antidepressants

Serotonin Reuptake Inhibitors (SSRIs) are primarily indicated for depression, but are also prescribed to treat compulsive behaviour, and eating or personality disorders. There are currently five SSRIs available on the market: citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline. Furthermore, several researchers detected fluoxetine in water bodies and municipal effluents as a consequence of excretion in unchanged form or improper disposal of unused medications (Daughton and Ternes, 1999). Kolpin et al.

(2002) estimated maximum fluoxetine concentrations at 0.012 mg L^{-1} in surface waters. Weston et al. (2001) detected fluoxetine in municipal effluents at concentrations from 0.32 to 0.54 mg L^{-1}.

The toxicity of SSRIs to aquatic organisms is not well documented in the literature (Henry et al., 2004). For example, a study carried out by Brooks et al. (2003) on the toxicity of fluoxetine using aquatic vertebrates and invertebrates in standardized tests reported No Observable Effect Concentrations (NOEC) in concentration of 56 mg L^{-1} for the reproduction in *Ceriodaphnia dubia*. Moreover, results of several unpublished acute toxicity tests indicate toxicity of fluoxetine HCl and paroxetine HCl in a range from 0.9 to 3.0 mg L^{-1} as concentration that affect (mortality and unspecified effects) 50% of test organisms (Webb, 2001). Therefore, this work evaluated fluoxetine (Prozac™) as representative compound of the SSRI antidepressants.

1.8.5. Antiepileptics

The antiepileptic carbamazepine is an established drug for the control of psychomotor epilepsy, it is also effective in the treatment of trigeminal neuralgia and it is used in bipolar depression. Carbamazepine is predominantly metabolized in the liver, and at least 30 different metabolites have been identified (Lertratanangkoon and Horning 1982). Only 2–3% of the given dose is excreted in unchanged form (Clara et al., 2004). Furthermore, carbamazepine is commonly detected in STP effluents in Germany at concentrations up to 6.3 mg L^{-1} and also is detected in surface water (Heberer and Stan, 1998), groundwater (Heberer, 2002) and drinking water (Hummel et al., 2006).

Additionally, there are some reports that shown the toxicity on aquatic organisms due to carbamazepine presence. For example, Thaker (2005) reported acute toxicity of carbamazepine in *Daphnia* at 17.2 mg L^{-1} and at 34.4 mg L^{-1} in midges. Moreover, traditional toxicity tests performed by Ferrari et al. (2003) showed chronic toxicity of carbamazepine in *C. Dubia* [NOEC (7 days) = 25 g L^{-1}], in the rotifer B. Calyciflorus [NOEC (2 days) = 377 g L^{-1}], and in early life stages of zebrafish [NOEC (10 days) = 25 g L^{-1}]. As a consequence of the recalcitrant behaviour and potential effect of carbamazepine in aquatic systems, this compound was evaluated in this work.

1.8.6. Tranquillizers

Tranquilizers are drugs prescribed for anxiety, depression and insomnia, the most common form of tranquilizers is a group called benzodiazepines. This group is one of the most used classes of drugs in the world, with diazepam being the most well known compound (Van der Ven et al., 2004). In Denmark, diazepam is listed as one of the top 20 most used pharmaceuticals (Stuer-Lauridsen et al., 2000). Moreover, diazepam is almost completely transformed into its main metabolite desmethyldiazepam and to a minor extent to temazepam and oxazepam which are excreted primarily in the urine conjugated as glucoronides (Klotz, 1977). However, Ternes (1998) reported diazepam in 8 of 20 STPs in Germany but only in low concentrations less than 0.04 µg L^{-1}. Moreover, Fent et al. (2006) reported detection of diazepam in influents up to 1.2 µg L^{-1} and in effluents of 0.7 µg L^{-1} of STPS, whereas in Belgium diazepam was found at concentrations up to 0.66 g L^{-1} (Van der Ven et al., 2004).

On the other hand, diazepam like carbamazepine is classified as potentially harmful to aquatic organisms because most of the acute toxicity data are below 100 mg L^{-1} (Fent et al., 2006). Some studies on the potential effect of diazepam were reported by Calleja et al. (1994) that determined the LC_{50} (24h) in *D. Magna* in a range of concentrations between 9.6 mg L^{-1} and 1000 mg L^{-1}. Moreover, Pascoe et al. (2003) reported that in a study using cnidarian *Hydra vulgaris*, diazepam showed an inhibition of polyp regeneration at concentrations of 10 g L^{-1}. As a consequence of the potential effect and persistent behaviour in aquatic systems, diazepam was chosen for this study.

1.8.7. Fragrances

Fragrances are perhaps the most widely studied class of personal care products that are ubiquitous, persistent and bioaccumulative pollutants that are sometimes highly toxic. The most common fragrances are synthetic musks used in a wide-range of products such as the active ingredients or preservatives in cosmetics, toiletries, or fragrances. Synthetic musks are either nitro musks, which were introduced in the late 1800s, or polycyclic musks, introduced in the 1950s (Daughton and Ternes, 1999). The polycyclic musks celestolide (CEL), galaxolide (GLX) and tonalide (TON) are the major musks used today in high quantities. GLX and TON production alone has been

estimated to be about 1 million pounds per year and thus has been placed on the High Production Volume List by the USEPA (Peck, 2006). Polycyclic musks have been observed more often in surface water, in 78.3% for TON and 84.6% for GLX of samples, as well as in greater concentrations than nitro musk compounds worldwide (Brausch and Rand, 2011).

Polycyclic musks fragrances contained a high octanol–water coefficient between log K_{ow} 4.6 to 6.6 (Suarez et al., 2008), that indicate a high potential for bioaccumulation in aquatic species (Geyer et al., 2000). This potential has been studied by numerous researchers that identified high concentrations of musks in lipids from fresh- and saltwater fish and molluscs (Schramm et al., 1996). According to the potential effect of these compounds, this work was focused on three polycyclic musk fragrances GLX, TON andCEL.

Table 1.6. Physico-chemical characteristics and biodegradability of selected organic micropollutants.

Category	Compound	Notation	MW	S [mg·L^{-1}]	H [atm·m^3·mol^{-1}]	pKa	log K_{ow}	log K_d	k_{biol} [L·g^{-1}SS·d^{-1}]
EDCs	17α-ethinylestraidol	EE2	296	4.8 - 11.3	$7.9{\cdot}10^{-12}$	10.5-10.7	2.8 - 4.2	2.3 – 2.8	7-9
Pharmaceuticals	Carbamazepine	CBZ	236	17.7	$1.1{\cdot}10^{-7}$	7.0-13.9	2.3 - 2.5	0.1 – 1.7	< 0.01
	Diazepam	DZP	285	50	$1.5{\cdot}10^{-7}$	3.3-3.4	2.5-3.0	1.3	< 0.03
	Ibuprofen	IBP	206	21	$1.5{\cdot}10^{-7}$	4.9 - 5.7	3.5 - 4.5	0.9 – 1.4	9 – 35
	Naproxen	NPX	230	16	$1.4{\cdot}10^{-8}$	4.2	3.2	-	0.4-1.9
	Diclofenac	DCF	296	2.4	$1.9{\cdot}10^{-10}$	4.0-4.5	4.5-4.8	1.2	< 0.1
	Trimethoprim	TMP	290	400	$9.8{\cdot}10^{-13}$	6.6-7.2	0.9-1.4	2.3	-
	Roxithromycin	ROX	837	0.02	$1.0{\cdot}10^{-24}$	9.2	2.1-2.8	2.3-2.6	< 0.3
	Erythromycin	ERY	734	1.4	$2.2{\cdot}10^{-27}$	8.9	2.5-3.0	2.2	0.5-1
	Fluoxetine	FLX	346	60	$3.6{\cdot}10^{-6}$	10.1	4.05	-	-
Personal care products	Galaxolide	GLX	258	1.8	$1.3{\cdot}10^{-4}$	-	5.9 - 6.3	3.3 – 4.1	<0.03
	Tonalide	TON	258	1.2	$5.3{\cdot}10^{-3}$	-	4.6-6.4	3.4	<0.02
	Celestolide	CEL	244	0.22	$7.3{\cdot}10^{-1}$	-	5.4-6.6	3.9	-

MW: molecular weight; *s*: solubility in water (mg·L^{-1}); H: Henry coefficient (atm·m^3·mol^{-1}); pKa: dissociation constant; K_{ow}: octanol-water partition coefficient; K_d: sludge-water distribution coefficient; k_{biol}: pseudo first-order degradation constant (L·g^{-1}SS·d^{-1}); "-": data not available.US National Library of Medicine, Toxicology Data Network 2008 (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB); US National Library of Medicine, ChemIDplus Advanced 2008 (http://chem.sis.nlm.nih.gov/chemidplus); Liu et al. 2005, Penteado et al. 2006, Gómez-Gutiérrez et al. 2007, Ben et al. 2008, EFSA 2008, Kasprzyk-Hordern et al. 2008, Suárez et al. 2008.

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Chapter 2

Materials and Methods

ABSTRACT. - In this chapter, the analytical methods used in this work are described. It includes the physico-chemical parameters used for the wastewater characterisation, biomass characterisation present in different experimental set up and the analysis of Pharmaceutical and Personal Care Products, PPCPs.

From the conventional chemical parameters measured in the liquid phase the Total and Soluble Chemical Oxygen Demand (COD_T and COD_S), nitrite, nitrate and phosphorus and in the solid phase the Total, Inorganic and Volatile Suspend Solids (TSS, ISS and VSS) and the Settling Velocity were determined according to Standard Methods (APHA-AWWA-WPCF, 1999). Nitrogen in form of ammonium (NH_4^+), Total Organic and Inorganic Carbon (TOC, IC) and several inorganic anions (NO_2^-, NO_3^-, Cl^-, PO_4^{3-} and SO_4^{2-}) have been measured by analytical procedures optimised in our laboratories and these are thus described in throughout this chapter. Moreover, identification of the different populations present in the biomass samples was carried out by Fluorescent In Situ Hybridization (FISH).

A description of the analysis of PPCPs is also provided, including polycyclic musk fragrances (galaxolide, tonalide and celestolide), neutral pharmaceuticals (carbamazepine and diazepam), acid pharmaceuticals (ibuprofen, naproxen and diclofenac), anti-depressant (fluoxetine), estrogens (17α-ethinylestradiol), antibiotics (roxythromycin, sulfamethoxazol, trimethroprim and erythromicyn).

OUTLINE

2.1 LIQUID PHASE

In this section, the methods used for the determination of the conventional parameters of wastewater and sludge are described. For soluble fraction analysis, the samples were previously filtered with a pore size of 0.45 μm in order to remove suspended solids.

2.1.1. Carbon compounds

Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) is the amount of oxygen required to oxidise the organic matter present in a liquid sample (wastewater) using a strong chemical oxidant (potassium dichromate) in an acid medium. A catalyst (silver sulphate) is used to improve the oxidation of some organic compounds. After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulphate to determine the amount of $K_2Cr_2O_7$ consumed, being the amount of oxidable matter calculated in terms of oxygen equivalent.

The total and soluble Chemical Oxygen Demand (CODt and CODs) were determined following the method described by Soto et al. (1989), which is a modification from the method 5220C of the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1999). The difference between total and soluble COD is that CODt is determined using the raw sample, while for CODs determination, the sample is previously filtered through nitrocellulose-fiber filters (Whatman, GFC) with a pore size of 0.45 μm.

Reagents preparation

a) Standard potassium dichromate digestion solution: 10.216 g of $K_2Cr_2O_7$ and 33 g of $HgSO_4$ are dissolved in 500 mL of distilled water. Then, 167 mL of concentrated H_2SO_4 are added. The solution is cooled to room temperature and, finally, diluted to 1000 mL.

b) Sulphuric acid reagent: 10.7 g of Ag_2SO_4 are added to 1 L of concentrated H_2SO_4. The solution is used after 2 days of preparation.

c) Ferroin indicator solution: 1.485 g of $C_{18}H_8N_2{\cdot}H_2O$ (phenanthroline monohydrate) and 0.695 g of $SO_4Fe{\cdot}7H_2O$ are dissolved in 100 mL of distilled water.

d) Standard potassium dichromate solution 0.05 N. 1.226 g of $K_2Cr_2O_7$, previously dried at 105 °C for 2 hours, are dissolved in 500 mL of distilled water.

e) Standard ferrous ammonium sulphate titrant (FAS) 0.035 N: 13.72 g of $Fe(NH)_4(SO)_2 \cdot 6H_2O$ are dissolved in distilled water. Then, 20 mL of concentrated H_2SO_4 are added and, finally, the solution is cooled and diluted to 1000 mL.

Determination procedure

This procedure is applicable to samples with COD concentrations between 90-900 mg L^{-1}. Place 2.5 mL of sample in 10 mL Pyrex tubes. Add 1.5 mL of digestion solution and 3.5 mL of sulphuric acid reagent slowly on the wall of the tube slightly inclined (to avoid mixing). A blank sample using distilled water is prepared in the same way. This blank acts as "reference", representing the COD of the distilled water. After being sealed with Teflon and tightly capped, the tubes are finally mixed completely and placed in the block digester (HACH 16500-100) preheated to 150 °C. The duration of the digestion period is 2 h.

After digestion, the tubes are cooled to room temperature. Then, the content of the tubes is transferred to a beaker and, once added 1-2 drops of ferroin indicator, the solution is titrated under rapid stirring with standard FAS. The FAS solution is standardised daily as follows: Put 5 mL of distilled water into a small beaker. Add 3.5 mL of sulphuric acid reagent. Cool to room temperature and add 5 mL of standard potassium dichromate solution (0.05 N). Add 1-2 drops of ferroin indicator and titrate with FAS titrant. The end-point is a sharp colour change from blue-green to reddish brown. Molarity of FAS solution is calculated with the following equation (1):

$$M_{fas} = \frac{5 \cdot 0.05}{V_{fas}} \quad (1)$$

where M_{fas} is the molarity of FAS (mol L^{-1}), and V_{fas} is the volume of FAS consumed in the titration (mL).

The COD is calculated with the following equation (2):

$$COD = \frac{(A - B) \cdot M_{fas} \cdot 8000}{V} \quad (2)$$

where *COD* is the Chemical Oxygen Demand (mg O_2 L^{-1}), *A* are the mL of FAS consumed by the blank, *B* are the mL of FAS consumed by the sample, M_{fas} is the molarity of FAS (mol L^{-1}) and 8000 is milli-equivalent weight of oxygen x 1000 mL L^{-1}.

2.1.2. Total Carbon (TC), Total Organic Carbon (TOC) and Inorganic Carbon (IC)

Organic carbon in liquid samples may include a variety of organic compounds in different oxidation states. Total Organic Carbon (TOC) is a more convenient and direct expression of total organic content than COD, but does not provide the same information. Unlike COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganic that can contribute to the oxygen demand measured by COD (APHA-AWWA-WPCF, 1999). To determine the quantity of organically bound carbon, the organic molecules must be broken down and converted to a single carbon molecular form that can be measured quantitatively. The TOC concentration was determined by a Shimadzu analyzer (TOC-5000) as the difference between the Total Carbon (TC) and the Inorganic Carbon (IC) concentrations. The instrument is connected to an automated sampler (Shimadzu, ASI-5000-S). The TC concentrations are determined from the amount of CO_2 produced during the combustion of the sample at 680 °C, using platinum immobilised over alumina spheres as catalyst. The IC concentrations are obtained from the CO_2 produced in the chemical decomposition of the sample with H_3PO_4 (25%) at room temperature. The CO_2 produced is optically measured with a Non Dispersive Infrared Analyzer (NDIR) after being cooled and dried. High purity air is used as carrier gas with a flow of 150 mL min^{-1}. A curve comprising 4 calibration points in the range of 0 to 1 g C L^{-1}, using potassium phthalate as standard for TC and a mixture of sodium carbonate and bicarbonate (Na_2CO_3/$NaHCO_3$, 3:4 w/w) for IC, is used for the quantification (Figure 2.1).

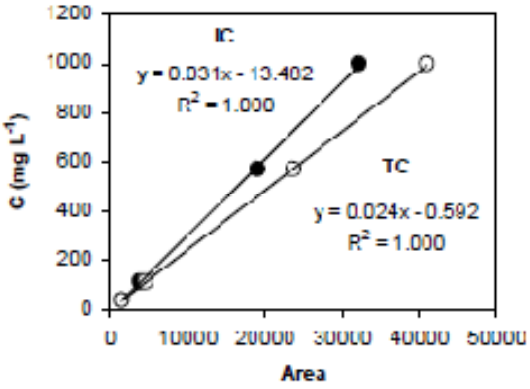

Figure 2.1. Calibration curve to determine TC and IC concentrations.

2.1.3. Ammonium nitrogen

Ammonium nitrogen is measured by a colorimetric method (Wheatherburn, 1967), based on the reaction of NH_3 with HClO and phenol, forming a strong-blue compound (indophenol) which can be colorimetrically determined using a spectrophotometer (Cecil CE 7200) at 635 nm.

Reagents preparation

a) Solution 1: Phenol-nitroprusiate, 15 g of phenol and 0.05 g of sodium nitroprusiate are added to 250 mL of buffer solution. The buffer solution was prepared adding 30 g of $Na_3PO_4 \cdot 12\ H_2O$, 30 g $Na_3C_6H_5O_7 \cdot 2H_2O$ and 3 g EDTA per litre, adjusted to pH 12.

b) Solution 2: Hipochloride, 15 mL of commercial bleach are mixed with 200 mL of NaOH 1 N and filled up to 500 mL with distilled water.

Determination procedure

Place 2.5 mL of sample (diluted if necessary to get a maximum concentration of 1 mg NH_4^+-N L^{-1}) and add, 1.0 and 1.5 mL of solution 1 and 2, respectively. After waiting 45 min at room temperature, the concentration of NH_4^+-N is measured in a spectrophotometer at 635 nm. The quantification is done with a 5-7 points calibration curve in the range of 0-1 mg NH_4^+-N L^{-1}, using NH_4Cl as standard (Figure 2.2).

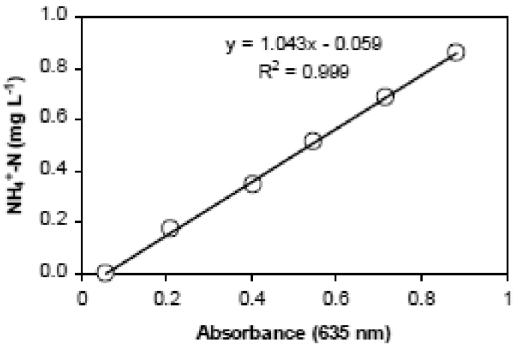

Figure 2.2. Calibration curve for ammonium concentration determination.

2.1.4. Nitrite

Nitrite concentration in wastewater is determined following the method 4500-NO_2^--B described in Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1999).

Nitrite is determined through the formation of a reddish purple azo dye produced at pH 2.0-2.5 by coupling diazotized sulphanilamide with N-(1-napththyl)-ethylenediamine dihydrochloride (NED dihydrochloride).

Reagents preparation

a) Sulphanilamide: 10 g of sulphanilamide are dissolved in 100 mL of concentrated HCl and 600 mL of distilled water. After cooling, the volume is filled up to 1 L with distilled water.

b) NED: 0.5 g of NED is dissolved in 500 mL of distilled water.

Determination procedure

To 5 mL of sample (diluted if necessary to fit the concentration range of the method), 0.1 mL of each solution (sulphanilamide and NED) are added. After waiting 20 min for colour stabilisation, the sample is measured in a spectrophotometer (Cecil CE 7200) at 543 nm. The quantification is done with 6-8 points calibration curve in the range of 0-0.30 mg NO_2—N L^{-1}, using $NaNO_2$ as standard (Figure 2.3).

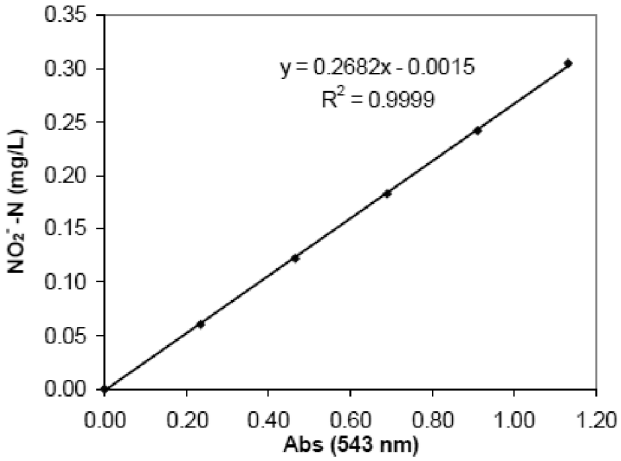

Figure 2.3. Calibration curve for nitrite concentration determination.

2.1.5 Nitrate

Nitrate concentration in wastewater is determined following the method 4500-NO3--B described in Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1999).

Measurement of UV absorption at 220 nm enables rapid determination of NO3- ions. Because dissolved organic matter also may absorb at 220 nm and NO^{3-} does not absorb at 275 nm, a second measurement at 275 nm is used to correct the NO_3^- value.

Determination procedure

Place 5 mL of sample (diluted if necessary to get a maximum concentration of NO_3^--N of 2.5 mg L^{-1}) and add 0.1 mL of HCl 1N. Afterwards, the absorbance at 220 and 275 nm is measured in a spectrophotometer (Cecil CE 7200). The absorbance related to nitrate is obtained by subtracting two times the absorbance reading at 275 nm from the reading at 220 nm. The quantification is done with a 6-8 points calibration curve in the range of 0-2.50 mg NO_3—N L^{-1}, using KNO_3 as standard (Figure 2.4).

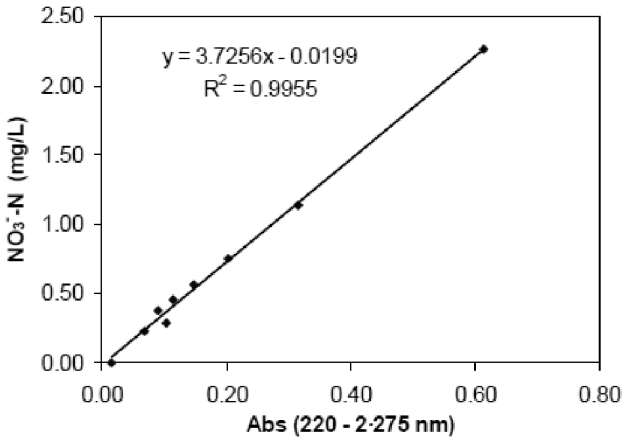

Figure 2.4. Calibration curve for nitrate concentration determination.

2.1.6. Phosphorous compounds

Phosphates

The method is based on the absorbance measurement at the radiation of 880 nm (Method 4500-PE of Standard Methods APHA-AWWA-WPCF, 1999).

Ammonium molybdate and antimony potassium tartrate react with orthophosphate in acid medium to form phosphomolybdic heteropolyacid. This compound is reduced by ascorbic acid into molybdate blue. The following reagents are necessary in order to carry out the procedure:

Reagent A: Sulphuric acid 5N.

Reagent B: Solution of antimony potassium tartrate. 1.3715 g of $K(SbO)C_4H_4O_6 \cdot 0.5H_2O$ are dissolved in 500 mL of distilled water. This solution must be kept in a bottle with glass top in order to be preserved.

Reagent C: Solution of ammonium molybdate. 20 g of $(NH_4)6Mo_7O_2 \cdot 4H_2O$ are dissolved in 500 mL of distilled water. This solution must be kept in a bottle with glass top in order to be preserved.

Reagent D: Ascorbic acid 0.01M. This solution is stable for one week.

Combined reagent: To prepare 100 mL of the combined reagent, the reagents A to D are mixed according to the following volumes: 50 mL of reagent A, 5 mL of reagent B, 15 mL of reagent C and 30 mL of reagent D. The mixture must be stirred after the addition of each reagent, following the mentioned order. This combined reagent is stable for 4 hours.

Determination procedure

A sample of 5 mL is taken and one drop of phenolphthalein indicator solution (0.5-1 g phenolphthalein in 1 L of ethanol at 80% concentration) is added. If red colour appears, reagent A (H_2SO_4 5N) is added (drops) until the red colour disappears. Then, 0.8 mL of the combined reagent is added and the mixture is stirred with a vortex stirrer. After 10 minutes but before 30 minutes, the absorbance at 880 nm is measured with a spectrophotometer Cecil CE 7200. A blank with reagents must be also measured as a reference.

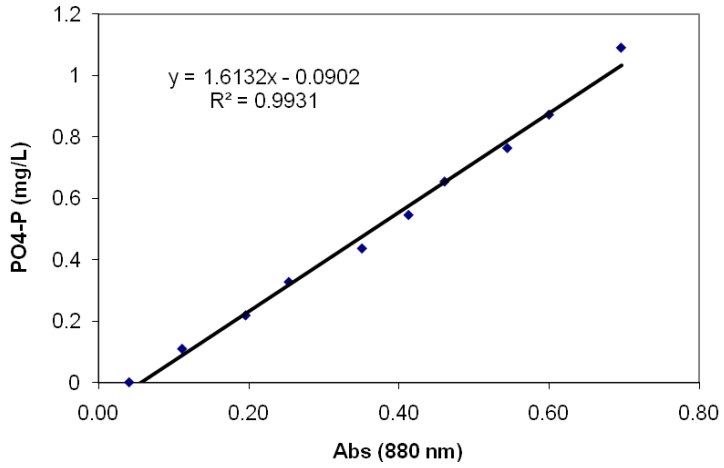

Figure 2.5. Calibration curve for phosphates concentration determination.

2.1.7. Inorganic anions

Nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) are determined simultaneously by capillary electrophoresis using a Waters Capillary Ion Analyzer (CIA). Sodium sulphate (0.01 M) is used as electrolyte (Vilas-Cruz et al., 1994). Besides, an electro-osmotic modifier (50 mL L^{-1}) CIA-Pak™ OFM Anion BT Waters (Ewing et al., 1989) is also added. The sample is forced to migrate through a capillary (melting silica covered with poliimida, 60 cm long and 45 μm of internal diameter) kept at 25 °C by the application of an electric current. Depending on the ratio charge/mass of the ion, the migrating time is different. A hydrostatic injection (10 cm height for 30 seconds) and an indirect detection (UV, 254 nm, 240 kV, 16-22 μA) are used.

Four to six calibration points for each ion in the range of 3-100 mg L^{-1} are daily used for the quantification of the samples (Figure 2.6). Previously to the analyses, the samples are filtrated through 0.45 μm membrane (Millipore).

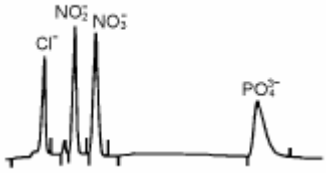

Figure 2.6. Typical electropherogram.

2.2 OTHER CONTROL PARAMETERS

2.2.1. pH

The pH is one of the key parameters measured in wastewater treatment systems, since its control is important to maintain the biological activity of the microorganisms involved in the treatment process. The pH measurements were performed with an electrode (Crison Instruments, S.A., 52-03) equipped with an automatic compensatory temperature device (Crison Instruments, S.A., 21-910-01) and connected to a measure instrument (pH mV-1). The sensibility of the instrument is ±1 mV, corresponding to 0.01 pH units. The electrode is calibrated at room temperature with two standard buffer solutions of pH 7.02 and 4.00.

2.2.2. Dissolved oxygen (DO)

A dissolved oxygen probe (AQUALITYC, model OXI-921) connected to a meter (M-Design Instruments TM-3659) was used to control DO concentration in the reactor.

2.2.3. Temperature

Temperature was measured using the same equipment as for the DO measurement.

2.3. SOLID PHASE

2.3.1. Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

Solids present in water can be organic or inorganic. Total Solids (TS), Total Suspended Solids (TSS), Volatile Solids (VS) and Volatile Suspended Solids (VSS) are determined following the methods 2540B, 2540D and 2540E,

respectively, described in Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1999).

Determination procedure

TS are determined weighing a selected (in order to yield a residue between 2.5 and 200 mg) well-mixed sample volume in a previously clean (heated to 103-105 °C for 2 h) dish after being evaporated at 103-105 °C until constant weight. The increase in weight over that of the empty dish represents the total solids in the initial volume of sample.

For the determination of TSS, a selected (in order to yield a residue between 2.5 and 200 mg) well-mixed sample volume is filtered through a weighed glassfiber filter (Whatman, GF/C, 4.7 cm of diameter, 1.2 μm of pore size) and the residue retained on the filter is dried to a constant weight (2h) at 103-105 °C. The increase in weight of the filter represents the total suspended solids.

To determine the volatile solids (VS or VSS), the residue from method 2540B and 2540D is burnt to constant weight at 550 °C during half an hour. The weight lost during ignition corresponds to the volatile solids. Since only a small amount of inorganic salts are decomposed and volatilised at that temperature.

This determination is useful in the control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge or industrial wastes.

2.4 BIOMASS CHARACTERISATION

2.4.1. Sludge Volumetric Index

SVI is typically used to monitor settling characteristics of activated sludge and other biological suspensions. The suspended solids concentration of a well-mixed sample (1 L of sample from the aerobic chamber) was determined. The 30 min settled sludge volume was determined.

The formula for SVI calculations:

$$SVI = \frac{1000 x SV}{SS} \quad (3)$$

where SV is the settled sludge volume (mL L^{-1}) and SS are the suspended solids (mg L^{-1})

Determination procedure

By means of observation and a stopwatch the volume of the settlement was measured. The measurement was noted at 1 minute intervals up to 6 minutes and thereafter every 3 minutes for the remainder of the 30 minute session.

2.4.4. Microbiological determinations

Identification of bacteria populations by FISH

The abundance of the different populations of microorganisms present in the sludge samples of the reactors was researched by Fluorescent In Situ Hybridization (FISH). With this technique specific regions in 23S or 16S rRNA are detected with fluorescently labelled probes. If the corresponding domain, phylum, genus or species is present, the probe hybridizes to the targeted sequence and can later be detected microscopically.

According to Amann et al. (1995) a typical FISH protocol includes the following steps (Figure 2.9): the fixation and permeabilization of the sample; hybridization of the targeted sequence to the probe; washing steps to remove unbound probe; and the detection of labelled cells by microscopy or flow cytometry. This protocol must be applied to disrupted biomass; therefore, the granules must be disintegrated before starting the procedure. To achieve the granular biomass breakage, biomass is sonicated for 1 min at 65% of amplitude using a probe sonicator (UP200s, Dr. Hielscher).

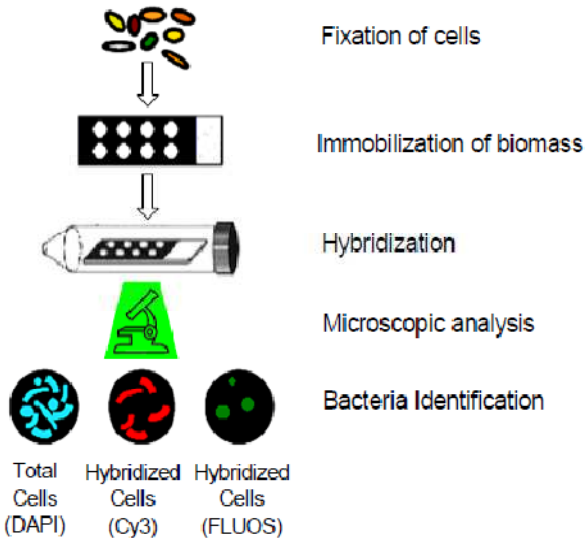

Figure 2.9. Different steps of the typical FISH protocol

During hybridization the cells are exposed to high temperatures, detergents and osmotic gradients. Thus fixation of the cells is essential in order to maintain the morphological integrity of the cells. Fixation of cells with glutaraldehide results in considerable autofluorescence of the specimen. Autofluorescence is minimized by fixation in freshly prepared (not older than 24 h) 4% paraformaldehyde solution in PBS.

After fixation, the cells are immobilized on a microscopic slide and used for hybridization with 16S rDNA probes. In order to avoid non-specific binding of the rDNA probes, the hybridization is done at stringent conditions (46 °C, 0-65% formamide) and specimens are washed with wash buffer (48 °C). The targeted organisms can be detected by the characteristic fluorescence.

The fluorochromes used to detect the hybridized rRNA were FLUOS (5(6)-carboxyfluorescein-Nhydroxysuccinimide ester) and Cy3 (indocarbocyanine). To visualize all cells in a sample the stain 4,6-diamidino-2-phenylindole (DAPI) was used. Its application can provide insight into the existence of archaeobacteria and eukaryotes, like e.g. protozoa. For analysis of the slides an epifluorescence microscope (Axioskop 2 plus, Zeiss) in combination with a digital camera (Coolsnap, Roper Scientific Photometrics) was used. The phylogenetic tree reflecting the different probes applied in this

study indicating the bacteria detected by each probe are shown in Figure 10. The probes applied in this study are listed and detailed in Table 2.1.

The probe for the domain of eubacteria (EUB338) was applied in all samples to get an impression of the relative abundance of the microorganisms detected by more specific probes. In comparison with DAPI they provided evidence of non-eubacteria present in the sample.

For further discussion it has to be kept in mind that samples can never be 100% representative. Thus the fact that no bacteria of a certain kind were present in the sample can always be attributed to unrepresentative sampling as well. Still it was tried to keep this error small.

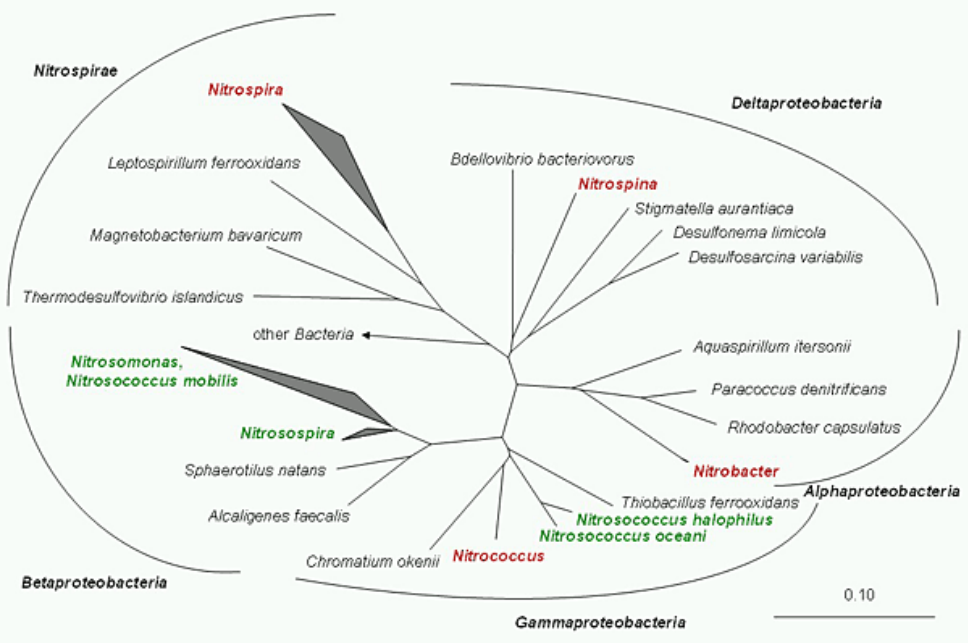

Figure 2.10. Different probes applied and the main bacteria detected by each probe (AOB: ammonium-oxidizing bacteria, NOB: nitrite-oxidizing).

Table 2.1. Probes used for fluorescent in situ hybridization and the formamide (FA) concentration used during hybridization.

Probe	Sequence (5'→ 3')	% Formamide	Target organisms	Reference
EUB338I	GTC GCC TCC CGT AGG AGT	20	Bacteria domain	Amann et al., 1995.
ALF1B	CGT TCG YTC TGA GCC AG	20	α-protobacteria, some δ-protobacteria, Spirochaetes	Manz et al., 1992.
BET42a	GCC TTC CCA CTT CGT TT	35	β-protobacteria	Manz et al., 1992.
NEU653	CCC CTC TGC TGC ACT CTA	40	Most halophilic and halotolerant *Nitrosomonas* spp.	Wagner et al., 1995.
NIT3	CCT GTG CTC CAT GCT CCG	40	*Nitrobacter* spp.	Wagner et al., 1996.
Ntspa712	CGC CTT CGC CAC CGG CCT TCC	50	Most members of phylum *Nitrospira*	Daims et al., 2001.
Pae997	TCT GGA AAG TTC TCA GCA	0	*Pseudomonas* spp.	Amann et al., 1996.
Nso1225	CGC CAT TGT ATT ACG TGT GA	35	β-protobacteria ammonia-oxidizing bacteria	Mobarry et al., 1996.
Nsv443	CCG TGA CCG TTT CGT TCC G	30	*Nitrosospira* spp.	Mobarry et al., 1996.
PAO651	CCC TCT GCC AAA CTC CAG	35	Most members of the Candidatus "Accumulibacter" cluster	Crocetti et al., 2000.
PAO462	CCG TCA TCT ACW CAG GGT ATT AAC	35	Candidatus "Accumulibacter phosphatis"	Crocetti et al., 2000.
PAO0846	GTTAGCTACGG CACTAAAAGG	35	Candidatus "Accumulibacter phosphatis"	Crocetti et al., 2000.

2.5. PPCPs

2.5.1. Selection of PPCPs

The complete list of PPCPs selected for the different experimental setups have included pharmaceuticals from five different therapeutic classes

(antibiotics, anti-depressants, anti-inflammatory drugs, tranquillisers and anti-epileptics), as well as three polycyclic musk fragrances, usually known in the form of cosmetic ingredients. The concentration of each substance was prepared according to Table 2.2.

Table 2.2. Theoretical concentrations of PPCPs spiked to each system feeding (C_{feed} in μg L^{-1}).

Compound	C_{feed}
Anti-depressant	
Fluoxetine (FLX)	20
Anti-inflammatories	
Ibuprofen (IBP)	10
Naproxen (NPX)	10
Diclofenac (DCF)	10
Anti-epileptic	
Carbamazepine (CBZ)	20
Antibiotics	
Trimethoprim (TMP)	10
Roxithromycin (ROX)	10
Erythromycin (ERY)	10
Tranquillizer	
Diazepam (DZP)	20
Musk	
Galaxolide (GLX)	40
Tonalide (TON)	40
Celestolide (CEL)	40

To prepare the samples, each substance was initially dissolved in either acetone or methanol and stored in a glass vial. Mixtures of these were then prepared according to the abovementioned concentrations. The bacteria were given time to adapt to the new system prior to "spiking". A predominance of nitrite/nitrate forms of nitrogen in the wastewater indicate that the waste has been stabilised with respect to oxygen demand (Metcalf and Eddy, 2003). Once conditions were found to be stable (namely, efficient removal of COD and ammonium) in the reactor, the selected PPCPs were added to the system using glass pipettes.

2.5.2. Sample preparation and Analysis of PPCPs

The analysis comprised of filtration (if only the liquid phase is considered), sample extraction, derivatization (if necessary) and quantification of compounds. In order to avoid interferences caused by suspended solids,

1-1.5 L of raw samples (from the influent and effluent) were first pre-filtered through glass fibre filter (AP4004705, Millipore) and then was filtered through a 0.45 μm glass fiber filter (APFC04700, Millipore). Sample extraction consisted of Solid Phase Extraction (SPE), which is a separation process by which the analytes present in an aqueous sample are sorbed in a solid phase material and then desorbed by elution with an organic solvent. For some compounds, a derivatization step prior to the chromatographic analysis is needed to ensure the stability of the substance along the column. In order to increase the number of samples extracted, a SPE 24-Position vacuum manifold (Phenomenex, USA) was used (Figure 2.11). Liquid or Gas Chromatography coupled with Mass Spectrometry (LC-MS or GC-MS, respectively) was used for the final quantification.

Figure 2.11. Manifold used in the Solid Phase Extraction (SPE) step for simultaneous concentration of different samples.

The equipment consisted of a gas chromatograph Varian CP 3900 (Walnut Creek, CA, USA) equipped with an automatic injector (CP 8400) and connected to an ion trap mass spectrometer (Varian Saturn 2100). Separation was carried out using a capillary column (CPSIL-8 CB Low bleed/MS, 30 m x 0.25 mm d.i., DF: 0.25 μm). The carrier gas is Helium. Moreover, Agilent Liquid Chromatograph API 4000 G1312A equipped with a binary pump and an autosampler HTC-PAL was used. Separation was carried out with a column Phenomenex Sinergy 4u Max-RP 80A de 250 x 4.6 mm (particle size 4 μm) and the detection was performed with a triple quadruple Mass Spectrometer (MS) (Applied Biosystems, Foster City, CA). A binary gradient consisting of 0.1% formic acid (v/v) in water (A) and 100% methanol (B) at a flow rate of 700 μL min^{-1} was used. The gradient was as

follows: 5% B held for 3.5 min, increased linearly to 80% by 10 min and held for 3 min, and stepped to 100% and held for 8 min. A 9-min equilibration step at 5% B was used at the beginning of each run to bring the total run time per sample to 30 min. An injection volume of 5 µL was used for all analyses.

Table 2.3. Shows the transition of several target compound using ESI positive and the retention time.

Compound	Transition	Retention time (min)
SMX	254/156	13.4
SMX2	254/92.2	13.4
TMP	291.1/260.9	10.7
TMP2	291.1/230	10.7
ROX	837.37/679.3	13.9
ROX2	837.7/158.2	13.9
ERY	734.4/158.3	13.3
ERY2	734.4/576.3	13.3
FLX	310.1/44.1	13.6
FLX2	310.1/148.2	13.6

2.5.2.1. Neutral Pharmaceuticals and Polycyclic Musk Fragrances: Carbamazepine, Diazepam, Galaxolide, Tonalide and Celestolide

The SPE procedure (Figure 2.12) was used for the determination of the soluble load compounds in liquid samples. 250 ml are taken from the filtered sample and the pH adjusted to 2.5 with 1 N HCl. The following step consists of the addition of the surrogates (meclofenamic acid and dihydrocarbamazepine). The sample is concentrated in a 60 mg OASIS HLB cartridge previously conditioned with ethyl acetate, methanol and Milli-Q water to pH 2.5. Then, cartridges were dried completely by a nitrogen stream for 45 min. Once dry, it is eluted with 3 mL of ethyl acetate. This extract is divided into two aliquots. In one, PCB 30 (internal standard solution in ethyl acetate) is added and then analysed by means of Gas Chromatography connected to Mass Spectrometry (GC-MS). The limits of instrumental quantification are approximate (in ppb): GLX (2), TON (2), CEL (2), CBZ (40), DZP (20).

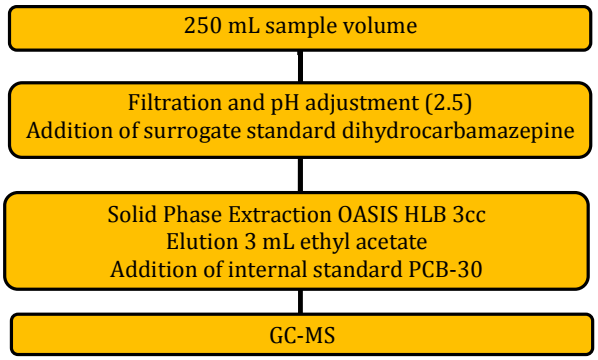

Figure 2.12. Scheme of the SPE method for musks and neutral pharmaceuticals

2.5.2.2. Acidic Pharmaceuticals: Ibuprofen, Naproxen and Diclofenac

The second aliquot of the extract obtained, as described in the previous section, was derived (sililation) by means of the addition of 200 µL MTBSTFA and heated at 60 °C for 60 min. Afterwards, PCB 30 is added and then analysed by GC-MS. This methodology for the acid analysis is based on the work of Rodriguez et al. (2003). The quantification of the different compositions was carried out by means of calibration taking into account the surrogates and the internal standard PCB-30. The limits of instrumental quantification for S/N=10 are approximate (in ppb): IBP (2), NPX (2) and DCF (8). The limits of quantification for the sample depend on the matrix analysed, since this determines the degree of concentration that can be reached.

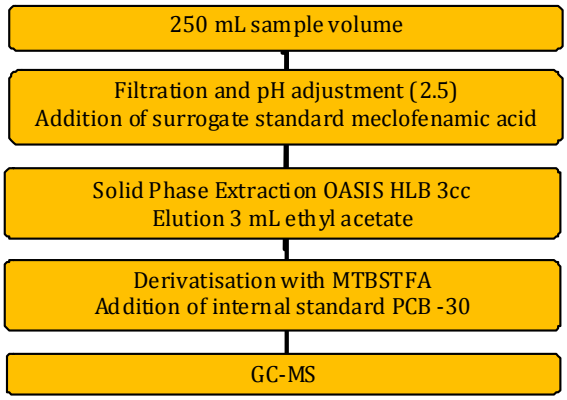

Figure 2.13. Scheme of the analytical method for acidic pharmaceuticals

The operation conditions of the GC-MS for neutral and acid compounds determination are summarised in Table 2.4.

Table 2.4. Operating conditions of GC and MS detection.

parameters	Fragrances and neutral compounds	Acid compounds
Injector *Split-Splitless*		
Splitless time	1 min	1 min
Inyector temperature	250 °C	280 °C
Gas flow (He)	1 mL min^{-1}	1 mL min^{-1}
Pressure pulse	30 PSI (1 min)	NO
Injector volume	1 µL	1 µL
Solvent	Ethylacetate	Ethylacetate
GC temperatures		
Initial temperature	60 °C	50 °C
Inicial time	2 min	1 min
1st ramp	10 °C min^{-1}	10 °C min^{-1}
Final temperature	250 °C	180 °C
Isothermal time	0 min	7 min
2nd ramp	20 °C min^{-1}	10 °C min^{-1}
Final temperature	280 °C	230 °C
Isothermal time	9.5 min	25 min
Rampa 3	--	20 °C min^{-1}
3rd Temperature	--	250 °C
Tiempo 3	--	5 min
MS parameters		
Ionization mode	EI	EI
Filament current	20 µA	10 µA
Ion trap temperature	220 °C	220 °C
Transfer line	280 °C	280 °C
Multiplicador voltage	1700-1750 V	1700-1750 V
Scan velocity	0,76 s $scan^{-1}$	1 s $scan^{-1}$
Mass spectrum	45-400 m z^{-1}	100 - 330 m z^{-1} (10–25 min) 140 - 420 m z^{-1} (25–57 min)
m z^{-1} quantification	GLX and TON (243) CEL (229)	IBP (263) NPX (287)

	CBZ (193+236) dihydrocarbamazepine (195 + 238) DZP (256 + 283) CB 30 (256 +258)	DCF (352+354+356) meclofenamic acid 352+354+356) CB 30 (256 +258)

2.5.2.3. Anti-depressants and Antibiotics: Fluoxetine, Roxithromycin, Trimethoprime and Erythromycin.

The chemical analysis for the determination of the concentration of fluoxetine (FLX), trimethoprim (TMP), roxithromycin (RXT) and erythromycin (ERY) was performed as published by Vanderford et al. (2003). 250 mL of filtered sewage or permeate/effluent samples were passed through an Oasis HLB 60 mg cartridge (approximately at 15–20 mL min^{-1}) that had been sequentially pre-conditioned with methanol, methyl tert-butyl ether and Milli-Q water adjusted at the same pH that the sample (3 mL each). The elution step was performed with 1.5 mL of a mixture of methanol: Methyl tert-butyl ether (10:90) and 1.5 mL of methanol (Figure 2.14). Final detection was performed in an LC-MS-MS in the positive ESI mode. Other procedures involving optimization of the MS, selection of best ionization source and mode for each analyte, source polarity and adjustments were carried out according to the method published by Vanderford et al. (2003). The limits of instrumental quantification are approximate (in ppb): FLX (0.1), ROX (0.1), ERY (0.1) and TMP (0.5).

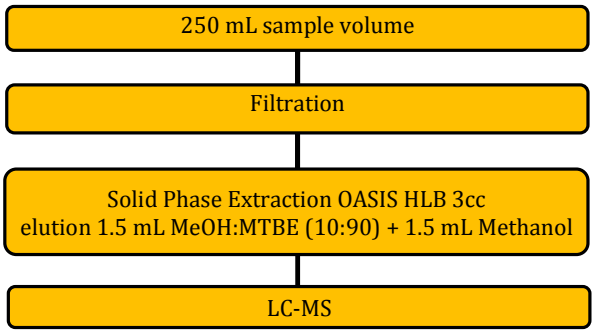

Figure 2.14. Scheme of the analytical method for anti-depressants and antibiotics.

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Chapter 3

Factors influencing adsorption of micropollutants on sludge[1]

ABSTRACT.- A new methodology, previously used to study partitioning of Polycyclic Aromatic Hydrocarbons (PAHs) (4.2>log K_{ow}>6.6) in complex heterogeneous systems such as mixed liquor in bioreactors was applied to the synthetic hormone 17α-ethinylestradiol (EE2), an organic micropollutant commonly present in sewage. This model considers the sludge as a three compartment matrix, in which the pollutant coexists in three states: freely dissolved, sorbed-to-particles and sorbed-to-dissolved and colloidal matter (DCM), being possible in this way to gain a better knowledge about the affinity of the pollutant for all of these phases, through the determination of the equilibrium constants K_{part} and K_{DCM}. According to the results, the measured partition coefficients related to EE2 are in the range of 315 to 439 L kg^{-1} (log K_d 2.5 – 2.64). The values of K_{part} and K_{DCM} determined by this model were 384 L kg^{-1} part and 45 L kg^{-1} DCM, respectively, which indicates that affinity to particles is the most relevant factor for this compound being the affinity to DCM poorly relevant.

[1]This chapter has been submitted as:

D. Serrano, J.M. Lema, F. Omil, D. Patureau. Application of a three-compartment model for 17α-ethinylestradiol as a tool to predict its sorption onto sludge.

OUTLINE

3.1. INTRODUCTION

The fate of many environmental contaminants, mainly those with hydrophobic character and low aqueous solubilities, is influenced not only by their interaction with the particulate fraction but also with dissolved macromolecules or colloidal particles dispersed in a given water compartment. In fact, the interaction between the pollutants and dissolved solutes and colloids may allow their transport within the water stream (Magee et al., 1991), as well as favouring their mobilization from the solid phase (sediments, soils or sludge). In this sense, Dissolved Organic Matter (DOM) has been reported to play an important role in transport of hydrophobic organic contaminants with groundwater and surface water since the presence of DOM, even at low concentrations, has been reported to have a significant effect on reduction of the sorption coefficient for a wide number of substances (Amiri et al., 2005).

Sorption of environmental pollutants, especially those hydrophobic organic chemicals, onto particulate matter, sediments, soils or even DOM, is often described with a linear sorption isotherm. However, non-linear isotherms have been also often reported, being those data usually described by the Freundlich model. Although non-linearity sorption has been observed in many studies, deviations from linearity are found particularly at high solute concentrations in water (Schrap and Opperhuizen, 1992).

According to different investigations dealing with micropollutants sorption, the sludge has been considered as a two-compartment model; however several sorption studies report data which contradict this model. Schrap and Opperhuizen (1992) indicate two important evidences which seem to contradict the partitioning model: i) the adsorption-desorption hysteresis and, ii) the sediment-water ratio effect. In order to explain these observations, both authors have proposed the “third phase model”, which is based on the assumption that it is impossible to achieve a complete separation between water and sludge. Depending on the separation technique and on the solid phase used, this incomplete separation means that parts of the solid phase (the third phase) remain in the aqueous phase. Because chemicals are sorbed in this third phase, the total concentration of the chemical in the aqueous phase is enhanced by the presence of the third phase material in the aqueous phase. Consequently, the total concentration in the aqueous phase which is determined experimentally can be given by the

sum of the concentration of the truly dissolved chemical and the amount of the chemical which is sorbed in the third phase.

The separation of particulate matter (aggregates that settle down due to gravity) and dissolved and colloidal matter (DCM, which comprises colloidal and truly dissolved matter) can be performed by cross-flow ultrafiltration at 0.5 μm, being the limit between colloidal matter and truly dissolved matter (tDM) around 20 nm. The more micropollutants are bound to tDM, the more bioavailable they are, which implies that it is necessary to investigate this fraction in order to evaluate the toxicological hazard linked to a water sample (Jeanneau et al., 2007).

Endocrine Disrupting Compounds (EDCs) comprises a diverse range of both natural and synthetic chemicals which can alter functions of the endocrine system and consequently cause negative adverse health effects on organisms (Johnson and Sumpter et al., 2001; McLachlan et al., 2006; Kidd et al., 2007). The contraceptive 17α-ethinylestradiole (EE2) is an organic micropollutant widely studied which has been reported to occur at very low concentrations, in the range of ng L^{-1}, in different environment compartments such as rivers, streams and surface waters as a consequence of the final discharge of the effluents of Sewage Treatment Plants (STPs) (Petrovic et al., 2002; Kidd et al., 2007). The behaviour and the removal of organic micropollutants during their passage through STPs depend on a number of factors, such as their physicochemical properties, STP configuration and operation, among others. Clara et al. (2004) reported that the higher removal pathways of EDCs are the sorption to sewage sludge, biological transformation and degradation.

EE2 is a compound with an intermediate lipophilic character (log K_{ow} of 2.8-4.2), and therefore it has been detected in water and sludge or soil compartments. Most of investigations related with EE2 sorption have been focussed on the use of the K_d values, which represents a two compartment model, i.e. the distribution of the pollutant among the aqueous phase and the solid phase.. Ternes et al. (2004) conducted a series of batch experiments using sewage sludge to determine the value of log K_d for EE2 equal to 2.5. Clara et al. (2004) found that the log K_d for EE2 was in the range of 2.71 to 3.0. Andersen et al. (2005) determined a distribution coefficient of 2.8 for EE2. However, taking into account previous considerations about the interaction of DOM and DCM with the fraction of pollutant present in the aqueous phase, as well as the contradictions discussed by Schrap and Opperhuizen (1992) recent works have proposed a three-compartment

matrix model to study micropollutants sorption equilibrium in sludge. In this way, Barret et al. (2010) applied and validated this three-compartment matrix model to a wide number of PAHs considering the three different phases in which the pollutant may occur (freely dissolved, sorbed-to-DCM and sorbed-to-particles) and calculating the different affinities for DCM and particles.

3.2. OBJECTIVES

The aim of this work is to study the suitability of this three-compartment matrix model used by Barret et al. (2010) to the organic micropollutant 17α-ethinylestradiol in order to gain a major insight about its affinity to solids and to DCM present in mixed liquor samples obtained from biological reactor commonly used in STPs.

3.3. MATERIALS AND METHODS

3.3.1. Activated sludge and DCM

The experiments were carried out using activated sludge from a domestic wastewater treatment plant, which was being operated at an hydraulic retention time in the aerated tank of 0.36 day. After collection, the activated sludge mixed liquor was stored at -20 °C in order to guarantee that all experiments were performed with the same matrix. Prior to experiments, the defrosted activated sludge was diluted 1:1 with tap water. DCM represents the sum of dissolved and colloidal matter, which corresponds to the defined supernatant obtained after centrifugation (12 000 rpm, 20 min and 35 °C) and filtration by a 1.2 µm filter. The DCM mass retained by this filter was negligible, since the DCM fraction beyond the size of 1.2 µm was insignificant.

Proteins were analyzed in accordance with Lowry et al. (1951) using Bovine Serum Albumin (BSA), whereas carbohydrates were determined using glucose (Glu) following the anthrone method (Dreywood, 1946). COD was determined with Merck Spectroquant kits, in accordance with the ISO 15705. Total Organic Carbon (TOC) in aqueous and solid samples was performed with Shimadzu analyzer (TOC-V_{CSN}). The lipid content in particles was defined as the Matter Extractable with Petroleum Ether (PEEM) at 70 bars and 105 °C with an Accelerated Solvent Extractor (ASE, Dionex).

3.3.2. Chemicals

The solvents (acetonitrile, methanol and acetone) were purchased from J.T. Baker (France). 17α-ethinylestradiol (EE2) powder was supplied by Sigma

Aldrich (purity >98%, St Quentin Fallavier, France). A concentrated stock solution was prepared at 5 g L^{-1} in acetonitrile and a final concentration solution of 0.5 g L^{-1} was prepared for spiking the samples. Both standard solutions were stored at -20°C.

3.3.3. Equilibrium time and Adsorption Isotherm

In a first experiment the sorption equilibrium time was determined. Prior to start the assay, the sludge (20 g_{TS} L^{-1}) was acclimatized by mixing during 2 h at room temperature. Then, 500 mL of sample of sludge was spiked with 1.29 mg EE2 L^{-1} to start with the sorption kinetics. Flasks were filled with 35 mL of activated sludge and shaken at 125 rpm at 35 °C. Samples were taken in regular time intervals (0.5, 1, 3, 6, 10, 24 and 48 h), the phases were separated by centrifugation (10 000 rpm at 35°C for 15 min) to give a clear supernatant and a pellet that was transferred to aluminium caps and kept at -20°C until ASE extraction. The clear supernatants were analyzed by HPLC.

For determination of the adsorption isotherm, 500 mL of sludge sample (20 g_{TS} L^{-1}) was acclimatized at room temperature. After 2 h, the sample was spiked with concentrations ranging from 0.2 to 1.8 mg EE2 L^{-1} and kept at 35°C for 5 h, according with the previously determined equilibrium time. The samples were then centrifuged at 35°C and C_{aqu} and C_{part} were measured according to the analytical section.

3.3.4. The three-compartment model

The compound studied can be present in the sludge in different compartments: freely dissolved (C_{free}, mg L^{-1}), sorbed-to-DCM (C_{DCM}, mg kg^{-1}_{DCM}), and sorbed-to-particles (C_{part}, mg kg^{-1}_{part}). At equilibrium, the three compartment system can be described by the two following equations:

$$K_{part} = \frac{C_{part}}{C_{free}} \text{ (1) and } K_{DCM} = \frac{C_{DCM}}{C_{free}} \text{ (2),}$$

where K_{part} is the equilibrium constant of micropollutant sorption to particles (L kg^{-1}_{part}) and K_{DCM} is the equilibrium constant of sorption of DCM (L kg^{-1}_{DCM}). C_{free} is very difficult to obtain experimentally whereas C_{aqu} (concentration of apparently dissolved micropollutant, sum of the freely dissolved and sorbed-to-DCM states, mg L^{-1}) and C_{part} can be easily determined experimentally. From C_{aqu} and C_{part} measurements, K_d (L kg^{-1}_{part}) can be estimated:

$$K_d = \frac{C_{part}}{C_{aqu}} \text{ (3)}$$

$$K_d = \frac{C_{part}}{C_{aqu}} = \frac{C_{part}}{C_{free}+C_{DCM}[DCM]} = \frac{K_{part}C_{free}}{K_{DCM}C_{free}[DCM]+C_{free}} = \frac{K_{part}}{K_{DCM}[DCM]+1} \quad (4)$$

where *[DCM]* represents the DCM concentration (kg_{DCM} L^{-1}). K_d is a partition coefficient, which depends on of three phases. Equation (4) was previously used in several studies dealing with the effect of DCM on micropollutant sorption in the environmental aquatic system (Schrap and Opperhuizen, 1992; Amiri et al., 2005).

A non linear regression algorithm of Levenberg-Marquardt type (Marquardt, 1963) was used to minimize the sum of square errors and to estimate the two parameters of the K_d model: K_{part} and K_{DCM}.

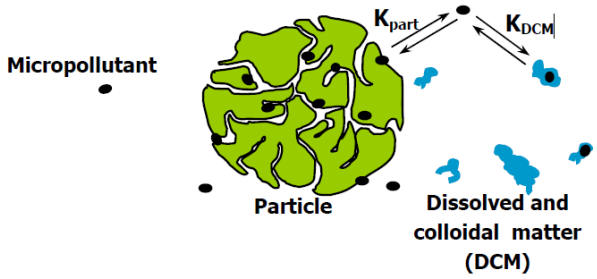

Figure 3.1. Representation of the three-compartment model of micropollutants in sludge (Barrret et al., 2010).

3.3.5. K_d, K_{part} and K_{DCM} determination

K_{part} and K_{DCM} can be obtained from equation (4) by assessing K_d with various DCM concentrations for EE2. According with Barret (2009), the experimental procedure comprised the following steps:

1. The defrosted activated sludge mixed liquor was firstly diluted 1:1 with tap water and Total Solids (TS) and Volatile Solids (VS) were determined according to standard methods (APHA, 1999).

2. 5 L of the diluted activated sludge were maintained during 2 h at room temperature.

3. 300 mL of this mixed liquor were then placed in a shaker at 120 rpm and 35°C, during 4 h.

4. After this time, centrifugation was applied (10 000 rpm during 20 min at 35°C).

5. Since during centrifugation some sludge particles do not adhere to the final sludge pellet obtained, a filtration using a 1.2 µm filter was performed to remove them.

Once determined the TS content of the diluted activated sludge mixed liquor and the supernatant obtained, it is possible to calculate by difference the TS content corresponding to the particles.

$$TS_{activated\ sludge} = TS_{DCM} + TS_{part} \quad (5)$$

In order to obtain a given concentration of DCM and particles to perform the tests, different dilutions of the activated sludge were carried out with distilled water and its own supernatant in different proportions. The final sludge samples obtained were spiked to achieve a concentration of 70 mg EE2 kg^{-1} TS in the solid phase and then stirred at 120 rpm and 35 °C during 5 h. Finally, centrifugation was applied in order to get the aqueous and solid fractions.

The EE2 quantification was carried out in the two phases, according with the following methodology:

• *Aqueous phase analysis:* Supernatants obtained were stored at 4°C in amber flasks until their measurement in the HPLC.

• *Solid phase analysis:* Pellets were transferred to aluminium caps and kept at -20 °C. Extraction from dried sludge samples were performed with an ASE-200 system (DIONEX™). The extraction solvent consisted of a mixture of methanol: acetone (50:50). The ASE cells were filled as follows (from bottom to the top): a filter of glass fiber (Diameter 19 mm, Whatman TM), 1 g of sodium sulphate pyrolized, 0.5-1 g of dried sludge sample and sodium sulphate pyrolized to complete the volume of the cell. After extraction, the obtained liquid was evaporated under nitrogen flow to dryness. The residue was then dissolved in certain volume of a mixture of acetonitrile: water (60:40) and was immediately analysed by HPLC.

Quantification of EE2 concentrations both in the liquid and solids-extracts were done by High-Performance Liquid Chromatography (HPLC). It consists of a Waters 600 LCD HPLC Pump, a fluorescence detector HP Waters 2475, fitted with a 10 µL external loop. Chromatographic separation was performed with a Lichrospere 100 RP-18 5 µL Lichrocart 250-4 (MERK) and

gradient elution by acetonitrile-water (60:40) a flow rate of 0.8 mL min^{-1}. The HPLC displays a total run time of 30 min.

3.4. RESULTS AND DISCUSSION

The main characteristics of the WWTP activated sludge diluted 1:1 were: 20 g TS L^{-1} ±0.5 (25% DCM, 75% particles); 33.2 g COD L^{-1} ± 0.6; 5 gTS-DCM L^{-1} ± 0.5; 0.28 g protein g^{-1} TS ± 0.5 (measured as equivalents of BSA); 0.37 g carbohydrates g^{-1} TS ± 0.9 (measured as equivalents of glucose); 0.167 g lipids g^{-1} TS ± 1.3; 0.281 g TOC g^{-1} TS and 0.256 g TOC g^{-1} TS-DCM.

3.4.1. Equilibration time

The sorption kinetics of EE2 was performed at 35 °C and 120 rpm; pH was measured but not controlled, being close to neutrality (6.5-6.7). Figure 3.2 shows the concentrations obtained in both the liquid and solid phases corresponding to the different sampling points. Total mass balances (sum of the amount recovered in the liquid and in the solid) indicate a range of recovery from 47 to 74% of the initial EE2 added. The sorption equilibrium time was established by the measured concentrations in aqueous and particulate phases, having been observed a fast initial adsorption (≈90 %) during the first 0.5 h. The equilibrium time was fixed at 5 h due to the fact that sorption behaviour during the 10 first hours was similar and after 24 h, possible degradation might occur. In our study, the equilibrium time obtained was shorter than others investigations (Clara et al. 2004; Ren et al., 2007) which reported equilibrium times in the range of 8-10 h. Taking into consideration all points, the ratio between the amount of EE2 measured in the liquid phase and the total amount (C_{aq}/C_{tot}) was 16% ± 1, being the value of K_d in the range between 315-389 L kg^{-1}.

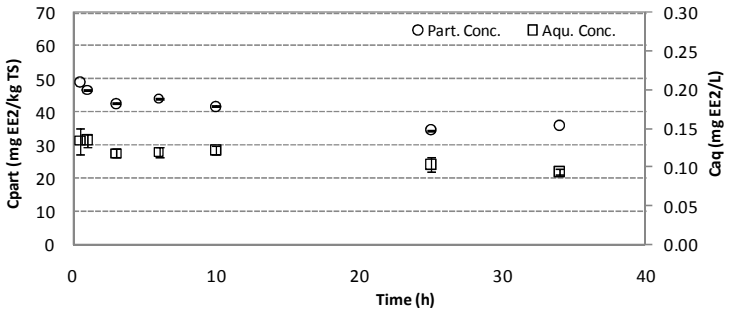

Figure 3.2. Aqueous and solid concentrations phase during sorption kinetics of EE2 at 35 °C with initial spike of 1.29 mg L^{-1}. The equilibrium time was achieved after 5h.

3.4.2. Adsorption isotherm

In this study the adsorption isotherm according to Freundlich was determined (Equation 6):

$$C_{part} = K_f \cdot C_{aqu}^{1/n} \quad (6)$$

where K_f and 1/n are the Freundlich adsorption coefficients. The graphical representation of the data obtained (Figure 3.3) shows that they can be successfully described by a line, thus indicating that under these conditions (high EE2 levels), the solid is far from being saturated (its maximum adsorption capacity). Once applied a linear regression, the values for the slope and the regression coefficient are: K_f=317.5 L kg^{-1} and r^2= 0.9995.

Total mass balances (sum of the amount recovered in the liquid and in the solid) indicate an average recovery of 86% ±12 of the initial EE2 added. The ratio C_{aq}/C_{tot} was 15% ± 1 and the value of K_d was in the range from 317 to 439 L kg^{-1}, values within the range of K_d previously reported for secondary sludge from 316 to 692 L kg^{-1} (Clara et al., 2004; Ternes et al., 2004; Andersen et al., 2005). The highest value of sorption capacity reached in this work was 69 mg EE2 kg^{-1} TS.

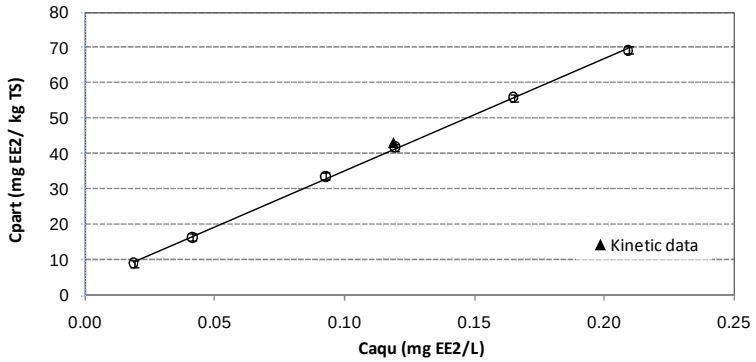

Figure 3.3. Adsorption isotherms of EE2 at 35°C, initial solutes concentrations ranged from 0.19 to 1.75 mg L^{-1} and contacting time: 5 h

As a conclusion, if we compare the results showed in Figure 3.2 and Figure 3.3, the value of K_d obtained in both assays are in the range from 315-439 L kg^{-1}.

3.4.3. K_d dependence to DCM concentration

The solid-water partition coefficient K_d corresponding to EE2 was determined as a function of the variation of the dissolved and colloidal matter concentrations. In accordance with the obtained results in the adsorption isotherms, a theoretical concentration of 70 mg EE2 kg^{-1} TS was spiked. Table 3.1 shows the different sludge samples prepared by adding different volumes of distilled water and supernatant.

Table 3.1: Experimental design for the study of K_d as a function of the DCM concentration (initial sludge characteristics: 20 g TS L^{-1} and 5 g TS-DCM L^{-1}).

Sample	V_{Sludge} (mL)	$V_{Supernatant}$ (mL)	V_{Water} (mL)	Sludge matter ($g_{TS,part}$ L^{-1})	DCM ($g_{TS,DCM}$ L^{-1})	Total Solids (g_{TS} L^{-1})
S1	20	0	50	4.4	1.5	5.8
S2	20	5	45	4.4	1.8	6.2
S3	20	10	40	4.4	2.2	6.6
S4	20	15	35	4.4	2.6	7.0
S5	20	20	30	4.5	2.9	7.4
S6	20	25	25	4.5	3.3	7.8
S7	20	30	20	4.4	3.7	8.1
S8	20	40	10	4.4	4.4	8.8
S9	20	50	0	4.4	5.1	9.5

Total mass balances in the experiment indicate a recovery of 115% ±9 of the initial EE2 added and the ratio between C_{aq}/C_{tot} was 40% ± 1, this ratio is higher than in the previous assays, due to the fact that at the maximum DCM concentration of 5.1 g L^{-1} (same as previously used), the C_{part} is now lower due to the dilution (4.4 g L^{-1}), far from the 15 g L^{-1} present previously. However, at this DCM concentration, the K_d value is 308, which is in agreement with the values obtained during the determination of equilibrium time and isotherms. Furthermore, EE2 measured in the solid and aqueous phases indicates that the K_d value varied in the range 311-355 L^{-1} kg (log K_d 2.50-2.55) showing a decreasing trend as we increase the DCM from 1 to 5 $g_{TS,DCM}$ L^{-1}.

Using the non linear regression algorithm of Levenberg-Marquardt type, the values of K_{part} (384 ±12 L $kg^{-1}{}_{PART}$) and K_{DCM} (45 ±11 L $kg^{-1}{}_{DCM}$) were estimated. Figure 3.4 shows the tendency of K_d values that decreased with

high DCM concentration.

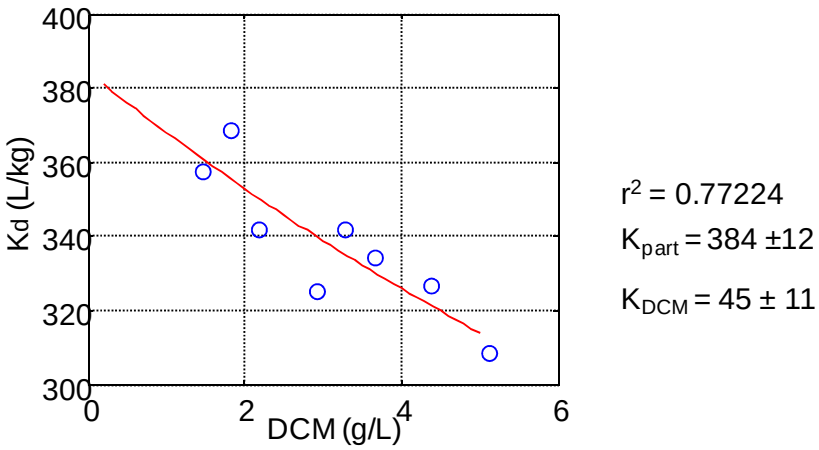

Figure 3.4. Measured (circle) and modelled (line) K_d (L kg^{-1}) as a function of DCM concentration (g L^{-1}), K_{par} and K_{DCM} were estimated using Levenberg-Marquardt algorithm.

According to the values of K_{part} and K_{DCM} obtained from the equation (4) with DCM ranging from 1.5 to 5 g_{DCM} L^{-1}, the EE2 affinity to DCM was very low compared with the particles affinity in the studied conditions. The range of DCM used in this experiment probably was not sufficient to observe a significant variation such as in the case of Barret et al. (2010) which used a DCM range between 0.6 and 4.5 g_{DCM} L^{-1}. The obtained partition coefficients could not be strictly compared to the literature data, because the studies about EE2 sorption published until now neither takes into account the three compartments nor the measurement of DCM concentration. However, in view of the fact that the higher affinity is with the particle phase, the reported values of K_{part} were in the range of the literature data (K_d 316-692 L kg^{-1}) which used a two compartment model.

Despite the results which did not reflected a significant interaction between our compound and the DCM, the methodology proposed by Barret et al. (2010) to study PAHS sorption equilibrium in sludge, considering sludge as three-compartment-matrix was transported and applied to EE2 as a useful tool to predict its fate and behaviour. The author proposes that the methodology could be applied to a wide range of micropollutants, mainly to hydrophobic compounds, where the DCM effect could be more noticeable. For example, Barret et al. (2010) tested a wide range of PAHs e.g. fluorene

which exhibits a K_{ow} 4.18 similar to EE2 (3.7-4.0) and K_d values ranging from 170 to 800 L kg^{-1} for DCM ranging from 0.6-4.5 g DCM L^{-1}, and with estimated K_{part} and K_{DCM} of 1606 and 1717 L kg^{-1}, respectively. These results indicated that the presence of DCM was significant and DCM interacts with this kind of compound: in this case the K_d value could be underestimated depending of the DCM level.

The results obtained in this study indicate that DCM is an additional factor that in the case of EE2 does not affect significantly the value of K_d. However, K_d is not a parameter that can be used as a unique value since it strongly depends on the operating characteristic of the STP. Other factors, as redox conditions, concentrations of micropollutants and temperature have also been pointed out in previous studies (Clara et al., 2004; Castiglioni et al., 2006; Suarez et al., 2008) as factors that affect the sorption behaviour of a compound.

3.5. CONCLUSIONS

A new methodology which considers the sludge as a three compartment matrix was applied using the synthetic hormone EE2 as a model compound. This substance is a well known organic micropollutant which has been detected in different environmental water compartments, particularly in discharges from sewage treatment plants. As a consequence of its intermediate hydrophobic character and log K_d values, a significant fraction has been detected associated with particles (sludge, sediments, soils, etc.).

This three compartment matrix model permits us to gain a major insight on EE2 partition since it takes into account not only the water and the particulate phases, but also the Dissolved and Colloidal Matter (DCM). In this sense, apart from the determination of the overall solid-water distribution coefficient K_d, two sorption equilibrium constants (K_{part} and K_{DCM}) can be determined through the use of this methodology, which give us detailed information about the interaction of this substance with particles and with DCM, respectively.

This work shows that when operating in the range of 1.5-5 g DCM L^{-1}, K_d values obtained for EE2 were 311-355 L kg^{-1} (log K_d 2.50-2.55), being the coefficients K_{part} and K_{DCM} obtained through the application of the three compartment matrix model 384 ± 12 L kg^{-1}_{PART} and 45 ±11 L kg^{-1}_{DCM}, respectively. Therefore, it is clear that for this compound the influence of the dissolved and colloidal matter is much less significant than the contribution

associated with its interaction with particles. A different behaviour with the conclusions reported for highly hydrophobic substances such as certain PAHs (Barret et al., 2010), for which K_{DCM} coefficients were more important, although being lower than K_{part} values. This research is considered of interest to be able to predict organic micropollutants behaviour in any sludge, as a function of its characteristics.

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Chapter 4

Removal of organic micropollutants from urban wastewater by an activated sludge system and hybrid activated sludge process[1]

ABSTRACT. - The performance of a lab-scale Hybrid Aerobic System (HAS) combining both suspended and fixed biomass for PPCPs removal was compared with the obtained by using a Conventional Activated Sludge (CAS) system. Both systems were able to achieve not only a high reduction of COD (>90%) and ammonium (>95%), but also high removal efficiencies for some PPCPs. Results indicate that HAS system exhibits an enhanced elimination for the acid compounds in the first sampling campaign with removals of 99% for IBP and 96% for NPX while in CAS system removals of 85% for IBP and 15% for NPX were reported. Although, medium removal efficiencies (70-78%) were found for DCF in CAS, a contradictory behaviour was observed in the hybrid system. More hydrophobic organic substances, like musk fragrances were removed almost completely in both reactors with a slightly higher removal in CAS. Furthermore, the anti-epileptic drug carbamazepine and tranquilizer diazepam displayed a high resistance to be removed in both reactors. In addition, FISH analysis determined the presence of ammonium oxidizing and denitrifying bacteria in the suspended biomass of HAS reactor. Despite the fact that HAS reactor worked by a short time of operation, these preliminary results indicate the potential influence of growing biofilm on a carrier as a way to enhance the presence of nitrifiers in the system and in this way, improving micropollutants removal.

[1]This chapter has been selected for a poster presentation:

D. Serrano, K. Lutchmiah, F. Omil, J.M. Lema. Removal of organic micropollutants from urban wastewater by an activated sludge system and hybrid activated sludge process.

OUTLINE

4.1. INTRODUCTION

Suspended growth processes are the biological treatment processes in which the microorganisms responsible for the conversion of the wastes are maintained in suspension within the liquid, whereas attached growth or fixed-film processes are biological processes in which the microorganisms responsible for the conversion of substrates in the wastewater are attached to some inert solid surfaces such as stones, sand, activated charcoal, metals, plastics sheets or foams (Gavrilescu and Macoveanu, 2000). A possible general classification of the different biological growth systems is shown in Figure 4.1. These processes are based on the use of two types of microbial aggregates: flocs and biofilms. The term floc refers to an assemblage of individual cells and micro-colonies occurring under specific reactor conditions (such as those imposed in activated sludge processes) or after the addition of various agents to the medium (Boonaert et al., 1999); whereas a biofilm can be defined as a complex coherent structure of cells and cellular products, like extra-cellular polymers (Donlan, 2002), which either form spontaneously as large, dense granules (Campos et al., 2009), or grow attached on a static solid surface (static biofilms) or on suspended carriers (particle supported biofilms) (Gavrilescu and Macoveanu, 2000).

A major disadvantages related to aerobic system is the important energy required to aerate wastewater and the high production of sludge (Méndez and Lema, 1992). In the case of fixed systems (trickling filters or submerged biofilters) the main drawbacks includes their relatively low volumetric removal rates (with consequently large sized biofilters), as well as biofilm shedding and risk of clogging when design or operation has not been adequate (Eding et al., 2006).

As a consequence, secondary treatment processes combining both fixed growth and suspended growth systems (Hybrid Systems, HS) are becoming very popular (Majumder and Gupta, 2003). Different works reported different advantages of the HS concept such as: i) higher biomass concentrations can be achieved with a wider microbial ecological diversity (Gavrilescu and Macoveanu, 2000); ii) enhancement of the nitrification efficiency, independent of the solids retention time of suspended biomass (Gebara, 1999); iii) simplicity of operation and higher process stability; iv) high effluent quality associated with suspended growth systems (Majumder and Gupta, 2003), and; v) the difusional barriers of the biofilm imply that the

biomass is less susceptible to irreversible damage due to the potential occurrence of shock or toxic loadings.

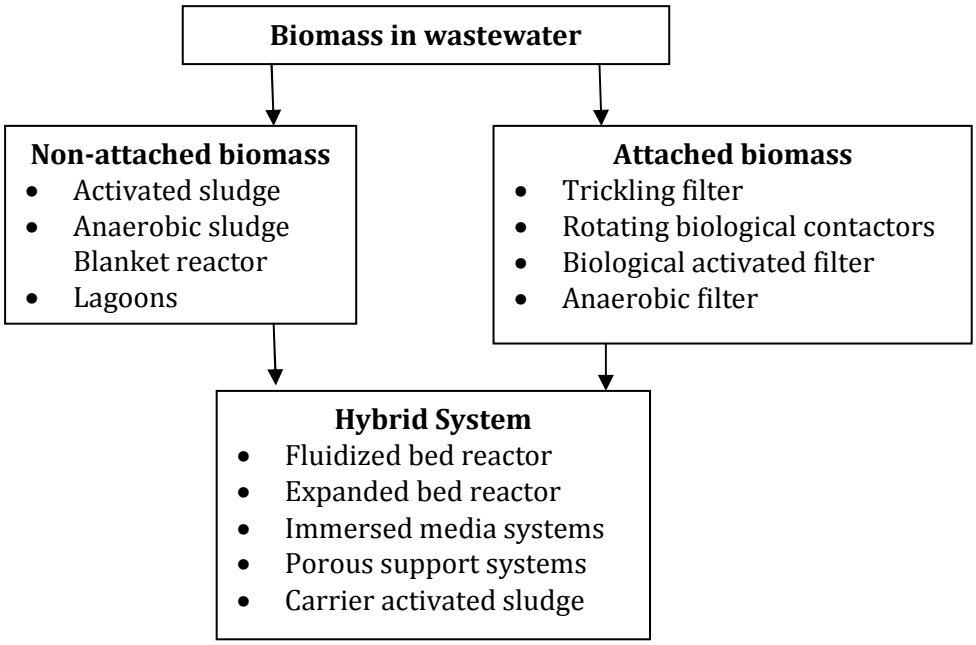


Figure 4.1. Classification of wastewater treatment bioreactors based on biomass attachment (adapted from Tyagi and Vembu, 1990).

In this context, the Moving Bed Biofilm Reactor (MBBR) concept is an example of a HS process (Buntner et al., 2010; Rusten et al., 2006). MBBRs are especially useful when slow growing organisms like nitrifiers have to be kept in a wastewater treatment process. Moreover, simultaneous nitrification and denitrification may take place in these reactors (Wang et al., 2006). MBBR is based on the use of suspended porous carriers kept in continuous movement in the aeration tank, while the active biomass grows as a biofilm on the surface of the carriers (Won-Seok Chang et al., 2002). The basic idea of MBBR is to maintain a continued operation biofilm reactor with a high density of biomass and without backwashing or sludge return (Li et al., 2011). Typical examples of activated sludge treatment process with suspended packing include Captor®, Limpor® and Kaldnes® supports (Buntner et al., 2010).

Previous studies carried out in MBBRs, using suspended carriers of polyethylene, reported successful removal of some toxic compounds such as phenol, thiocynate and ammonium in high concentrations. Moreover, these investigations proved that MBBRs can achieve higher removal rates due to the increase of biomass retained as biofilm and their wider diversity (especially in the slow growing nitrifiers). Besides, this reactor concept has been proven to exhibit a better resistance to shock loadings (Li et al., 2011; Borghei and Hosseini, 2004).

The application of biofilm systems to the removal of organic micropollutants such as PPCPs is still quite limited. Winkler et al. (2001) studied the biodegradability of ibuprofen and clofibric acid using a rotating annular reactors fed with raw river water as a sole source of carbon and nutrients. Their results indicate that ibuprofen was readily degraded (>90%) in biofilm reactor whereas clofibric acid elimination was negligible. Zwiener et al. (2003) using a lab-scale upflow biofilm reactor (anoxic-oxic) using pumice bed stones as support, studied the biodegradation of clofibric acid, ibuprofen and diclofenac. Their results showed all compounds were removed under anoxic conditions, obtaining lower values for ibuprofen (17-21%) and a bit higher for diclofenac (34-38%) as well as for clofibric acid (26-30%) whereas only ibuprofen was removed in the presence of oxygen (64-70%). Furthermore, Gunnarsson et al. (2009) evaluated the ability of six different sewage treatment processes, among of them the MBBR system, to remove estrogenic substances (steroid estrogens and phenol contained in industrial effluents) as a final polishing step of the effluent generated in a conventional activated sludge treatment plant. Their results indicated that MBBR system technology was effective in removal of these compounds (>99%).

4.2. OBJECTIVES

The aims of this work were: 1) to operate a Hybrid Aerobic System (HAS) using Kaldnes rings as support in order to study the removal of eight pharmaceutical and personal care products spiked in a synthetic medium-strength sewage, and 2) to compare the HAS performance with the PPCPs removal efficiencies obtained with a Conventional Activated Sludge system (CAS).

4.3. MATERIALS AND METHODS

4.3.1. Operation of reactors

The experiments were carried out using two laboratory-scale activated sludge plants (Figure 4.2), namely Conventional Activated System (CAS) and Hybrid Aerobic System (HAS). Each reactor consisted of 2 L aerated vessel coupled with a 1 L settler. In both reactors, recirculation of biomass from the bottom of the settler to the top of the reactor was made using a "mammoth pump" with air. The air flow rate provided oxygen to both units (> 6 mg L^{-1}) by distributors placed at the bottom of the aerated vessel in each reactor. The comparison between both reactors was made along the 60 days of operation of the HAS unit. Since no sludge purges were performed in both reactors, Sludge Retention Time (SRT) increased with the time of operation of each reactor.

CAS reactor was inoculated with 3.3 g VSS L^{-1} of aerobic sludge obtained from a full-scale municipal wastewater treatment plant whereas 2 L of activated sludge with 1.5 g SSV L^{-1} developed in a hybrid UCT reactor (Lutchmiah, 2009) was used to inoculate the HAS unit. The HAS moving bed biofilm reactor was filled with the biocarriers (K1 Anox-Kaldnes, Norway support rings; suspended polyethylene carriers). In order to permit the movement of the carrier freely in the aeration tank, only 40 % of the tank's capacity was filled with the supports (which correspond to a filling volume of approximately 0.8 L). The ratio of the volume of K1 rings in relation to the chamber volume is quite important (from 30 % to a maximum of 67 % is recommended by the manufacturer). Approximately 110 support rings were added to the system with the following characteristics: 1 g mL^{-1} density, 25 mm diameter, 10 mm wide and 350 $m^2 m^{-3}$ specific surface area.

Both reactors were previously operated with wastewater containing PPCPs. The composition of the synthetic feed (Table 4.1) has similar chemical characteristics as a medium-strength urban wastewater: 500 mg L^{-1} COD, 40 mg L^{-1} $N\text{-}NH_4$ and 8 mg L^{-1} $P\text{-}PO_4$. This solution was fed at a flow rate of 2 L d^{-1}, which led to a Hydraulic Retention Time (HRT) of 1 day.

Once steady state was achieved in both reactors, pharmaceuticals were spiked into the feeding tank from stock solutions of individual compounds (2000 mg L^{-1}) dissolved in methanol (ibruprofen, naproxen and diclofenac) or acetone (galaxolide, tonalide, celestolide, carbamazepine and diazepam).

Dilution water was added in order to ensure final concentrations commonly found in urban wastewaters (Table 4.2).

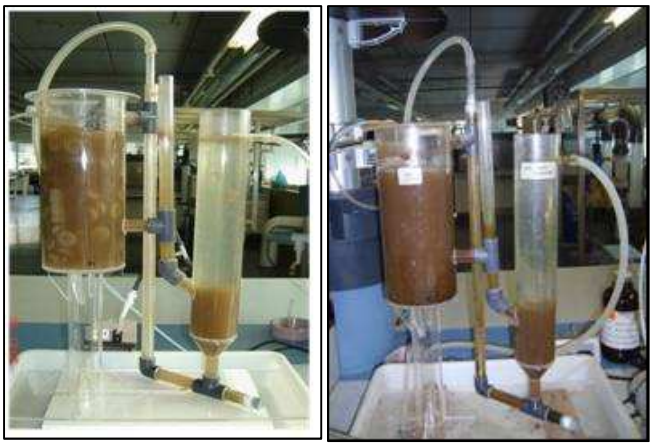


Figure 4.2. HAS (Hybrid Aerobic System) and CAS (Conventional Activated System).

Table 4.1. Composition of synthetic feed and trace solution.

Compounds in feeding	**Concentration (g L^{-1})**	**Compounds in the trace solution**	**Concentration (g L^{-1})**
$NaCH_3COO$	0.619	$FeCl{\cdot}6H_2O$	1.5
NH_4Cl	0.153	H_3BO_3	0.15
Na_2HPO_4	0.024	$CuSO_4{\cdot}5H_2O$	0.03
KH_2PO_4	0.012	KI	0.03
$NaHCO_3$	0.200	$ZnSO_4{\cdot}7H_2O$	0.12
Trace Elements*	0.5	$CoCl_2{\cdot}6H_2O$	0.15
		$MnCl_2{\cdot}4H_2O$	0.12

* Trace elements concentration is in mL L^{-1}.

Table 4.2. Theoretical concentrations of PPCPs spiked to CAS and HAS feeding (C_{feed} in µg L^{-1}), dissociation constant (pKa), octanol-water partition coefficient (log K_{ow}), solid-water distribution coefficient (K_d in Kg L^{-1}) and pseudo first-order degradation constant (k_{biol} in L g^{-1}SS d^{-1}).

Compound	C_{feed}	pKa	log K_{ow}	log K_d	k_{biol}
Anti-inflammatories					
Ibuprofen (IBP)	10	4.9-5.2	3.5-4.5	0.9[c]	9-35
Naproxen (NPX)	10	4.2	3.2	1.1[d]	0.4-1.9
Diclofenac (DCF)	10	4.1-4.2	4.5-4.8	1.2[c]	<0.1
Anti-epileptic					
Carbamazepine (CBZ)	20	7	2.3-2.5	0.1[c]	<0.01
Musks					
Galaxolide (GLX)	40	-	5.9	3.3[c]	<0.3
Tonalide (TON)	40	-	5.7	3.4[c]	<0.2
Celestolide (CEL)	40	-	5.4-6.6	3.9[f]	-
Tranquillizer					
Diazepam (DZP)	20	3.3-3.4	2.5-3.0	1.3[c]	<0.03

a) Jones et al. (2002); b) Brooks et al. (2003); c) Ternes et al. (2004); d) Urase and Kikuta (2005); e) Joss et al. (2005) and f) Kupper et al. (2006)

4.3.2. Analytical methods

Samples collected from CAS and HAS reactor were analysed for conventional physical–chemical parameters (COD, TSS, VSS, N-NH_4 and N-NO_3) according to standard methods (APHA, 1999). Moreover, biofilm content in the Kaldnes-support was determined by collecting the support media; weighed initially and then carefully cut into four pieces. Then, the pieces were placed in a vessel with 7 mL of distilled water and sonicated for 3 minutes (frequency 24 kHz, amplitude 208 µm and cycle operation 0.9 s/s). The system was maintained at low temperatures in a ice bath to avoid heating and disintegration of the particles. After the first sonification, the pieces were washed 3 times with distilled water, collected in an empty vessel introduced into a new vessel containing 7 mL of water and sonificated again. Thereafter, the liquid contents were placed into a crucible and follow the TSS and VSS methods.

Sampling campaigns were performed along each period of operation for the two systems in order to determine PPCPs in influent and effluent. Immediately after collecting 1 L in aluminium bottles, each sample was filtered through a 0.45 μm nitrocellulose filter. Analysis of Solid Phase Extraction (SPE) was used as pre-concentration technique prior to PPCPs quantitative determination. Liquid or Gas Chromatography coupled with Mass Spectrometry (LC–MS or GC–MS, respectively) was used for the final quantification. Analysis of the soluble content of anti-inflammatory compounds, carbamazepine (CBZ), diazepam (DZP) and musks were performed following the methodology described by Rodríguez et al. (2003), which consists of adjusting the pH of the samples to 2.5, adding meclofenamic acid and dihydrocarbamazepine as surrogate standards, carrying out a SPE of 250 mL samples using 60 mg OASIS HLB cartridges (Waters, Milford, MA, USA) and a final elution from the cartridge using 3 mL of ethyl acetate. This extract was divided into two fractions: one of them was used for direct determination of CBZ, DZP, GLX, TON and CEL, while the other one was employed for analysis of anti-inflammatories as their tertbutyldimethylsilyl derivatives. Finally, GC/MS detection was carried out in a Varian CP 3900 chromatograph (Walnut Creek, CA, USA) equipped with a split–splitless injector and connected to an ion-trap mass spectrometer.

4.3.3 Specific heterotrophic and nitrifying activity assays

Assays were carried out by means of a respirometric method (adapted from López-Fiuza et al., 2002) based on measurements of the oxygen concentration along time. These tests were performed using a Biological Oxygen Monitor (BOM, YSI model 5300) with oxygen selective electrodes (YSI 5331) connected to a data acquisition system (Figure 4.3). This system is a discontinuous respirometer that uses 15 mL vials with a maximum useful volume of 10 mL agitated by a magnetic stirring system and kept at a constant temperature of 25 °C by a thermostat.

Biomass is washed with phosphate buffer (Table 4.3) three times and, aerated during 30 minutes in order to remove completely the possible remaining substrate. Biomass is added to vials which are placed in a thermostatically controlled chamber at 25 °C provided with a magnetic stirring system. Air is used to reach the initial level of oxygen saturation. After an initial period of 2 minutes, the endogenous slope corresponding to oxygen consumption can be determined. Then, a certain amount of the required substrate is injected, which caused faster oxygen depletion,

reflected by a steeper slope. The reaction takes place until the time required to determine the rates of oxygen consumption (10-15 minutes). Finally, the content of biomass in vials is determined (TSS and VSS). The concentration of biomass used in this experiment remained in the range of 0.5 - 2 g VSS L^{-1}, and acetic acid was used as a substrate (concentration 400 mg COD L^{-1}) for the heterotrophic activity and ammonium (concentration 40 mg N-NH_4 L^{-1}) for nitrifying activity. All tests were performed by duplicate.

Table 4.3. Composition of phosphate buffer and trace metals solution (adapted from López-Fiuza et al., 2002).

Compounds	Concentration*	trace solution	Concentration*
KH_2PO_4	3.31	$FeCl{\cdot}6H_2O$	1,5
K_2HPO_4	3.97	H_3BO_3	0,15
$MgSO_4.7H_2O$	1.84	$CuSO_4{\cdot}5H_2O$	0,03
$MgCl_2.10H_2O$	1.52	KI	0,03
NaCl	0.80	$ZnSO_4{\cdot}7H_2O$	0,12
$NaHCO_3$	0.21	$CoCl_2{\cdot}6H_2O$	0,15
Traces	5.00	$MnCl_2{\cdot}4H_2O$	0,12

* All concentrations are in g L^{-1}, except the trace elements which are in mL L^{-1}.

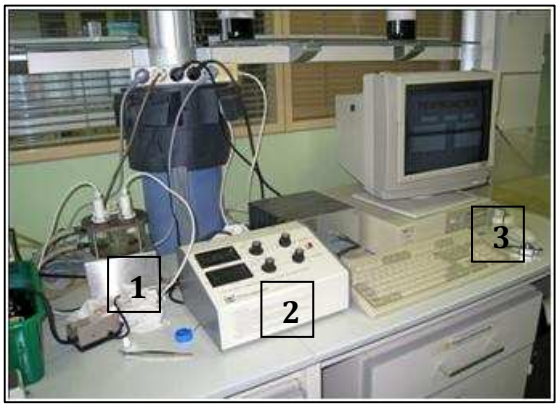


Figure 4.3. Respirometry system: 1) thermostatic test cells; 2) biological oxygen monitor; 3) data acquisition system.

To determine the activities in Kaldnes rings the same procedure as in the case of suspended biomass respirometry experiments was then carried out. Support rings were first weighed and then carefully cut into pieces (ensuring no disruption of the biofilm) enabling them to fit in DO vials. Thereafter the pieces were sonicated (Method for determining Biofilm) to verify the amount of VSS present.

4.3.4 Morphological biofilm observation and FISH analysis

Biofilm growth evolution on Kaldnes rings was monitored by stereo-microscope observation at magnifications 6.5X. Microbial populations were determined by Fluorescence In Situ Hybridization (FISH) technique. Biomass samples from both reactors were collected, disrupted and fixed, according to the procedure described by Amann et al. (1995), with 4% paraformaldehyde solution. Hybridization was performed at 46°C for 90 minutes adjusting formamide concentrations at different percentages (between 0-50%). The used probes for in situ hybridization were 50´ labelled with the fluorochromes FITC and Cy3. Fluorescence signals of disrupted samples were recorded with an acquisition system coupled to an Axioskop 2 epifluorescence microscope (Axioskop 2 plus, Zeiss). Probes EUB338, AlF1B, BET42a, NEU653, NIT3 and Pae997 were used.

4.4. RESULTS AND DISCUSSION

4.4.1. Operation of the CAS and HAS reactors

Two different configurations, a Hybrid Aerobic System (HAS) and a Conventional Activated Sludge (CAS) system at laboratory-scale were operated in order to compare their performance in terms of PPCPs removal. The main criteria for comparing both systems were: a) similar environmental and operational conditions applied; b) similar biomass concentrations in the mixer liquor and; c) reliability of PPCPs removal efficiencies obtained.

CAS reactor was previously operated with synthetic wastewater during several months under stable conditions. The support in the HAS reactor was added 30 days before this comparison took place in order to promote biofilm growth.

During the period of evaluation of both reactors, the environmental and operational conditions were maintained in the same range, as well as the composition of the influent (in terms of COD, N, P and micropollutants). The

main operational values of the conditions applied in each reactor are shown in Table 4.4.

Organic matter removal efficiencies were up to 90% in terms of soluble Chemical Oxygen Demand (COD) and the same behaviour was observed for ammonia nitrogen removal (>95%), with no significant differences between both systems (Table 4.4.).

During the evaluation period of both reactors, the specific activity in both suspended biomass of CAS reactor or biofilm of HAS reactor was determined in batch experiments. The specific heterotrophic activity and the specific nitrifying activity in the CAS reactor were 0.47 kg DQO kg^{-1} VSS d^{-1}, 0.007 kg N-NH_4 kg^{-1} VSS d^{-1}, respectively; whereas in the HAS unit were 0.27 kg DQO kg^{-1} VSS d^{-1}, 0.019 kg N-NH_4 kg^{-1} VSS d^{-1}, respectively.

Table 4.4. Performance of CAS and HAS systems during the micropollutants evaluation period (average values).

	HAS				CAS			
	S1	S2	S3	S4	S1	S2	S3	S4
HRT (d)	0.9	0.9	0.8	1.0	1.1	0.9	1.0	1.1
OLR (g COD L^{-1} d^{-1})	1.13	0.45	0.62	0.043	0.53	0.58	0.61	0.60
NLR (g N-NH_4 L^{-1} d^{-1})	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04
T °C	18	18	18	18	17	18	19	18
pH	7.2	7.8	8.0	8.5	8.5	8.3	8.4	8.4
DO (mg O_2 L^{-1})	8.0	7.8	8.0	8.5	8.5	8.3	8.4	8.4
VSS* (g L^{-1})	2.2	2.8	2.6	2.2	2.2	2.8	3.1	3.4
% COD removal	99	93	93	96	91	94	94	91
% N-NH_4 removal	97	98	98	98	97	95	95	95

* VSS corresponding to the suspended biomass in the mixed liquor.

4.4.2. Biofilm observation and FISH Analysis

Support inspection under optical microscopy shows the low biomass growth observed on the Kaldnes rings (Figure 4.4.). The comparison of the three pictures (taking on days 0, 24 and 46) permits to observe the development of a thin biofilm on internal and in a higher extent on external surfaces.

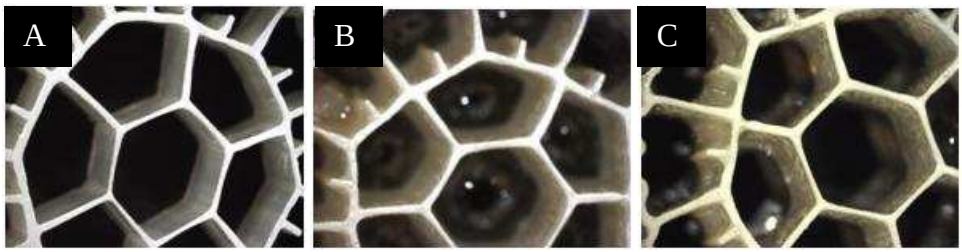


Figure 4.4. Microscopy photography of support rings in different days of operation of HAS system: A (day 0); B (day 24) and C (day 46). Magnifications 6.5X.

FISH technique was applied to detect microbial populations that could be present either in CAS or HAS reactor, according to the conditions and operation time in each reactor. Specific probes of bacteria were used to detect ammonia oxidizing bacteria *Nitrosomonas* (NEU653), nitrite oxidizing bacteria *Nitrobacter* (NIT3) and heterotrophic bacteria, *Pseudomonas spp.* (Pae997). Moreover, it is important to mention that a wide range of bacteria is able to carry out some of these metabolic processes and therefore, even though some probes that were used gave negative results, this means that another bacteria that carried out these processes could exist. The probes used in this work were chosen according to their availability and because most of the selected bacteria are commonly found in activated sludge systems.

In CAS and HAS systems a set of general probes, α-Proteobacteria (ALF1b) and β-Proteobacteria (BET42a), were applied in combination with the general Eubacteria domain probe (EUB3381) to detect the main bacteria involved in the process. The hybridized bacteria that belong to α-Proteobacteria (ie. *Nitrobacter*), β-Proteobacteria (ie. *Nitrosomonas spp*.) and *Pseudomonas spp*. class represent a small fraction in comparison with all the positives from EUB3381 probe.

In the case of the CAS system, probes for the identification of heterotrophic and denitrifying bacteria genus *Pseudomonas spp*. (Pae997) gave few positive results (Figure 4.5), whereas, no positive results were obtained with specific probes for Ammonia Oxidizing Bacteria (AOB) *Nitrosomonas spp*. (NEU653) and for Nitrite Oxidizing Bacteria (NOB) *Nitrobacter* (NIT3).

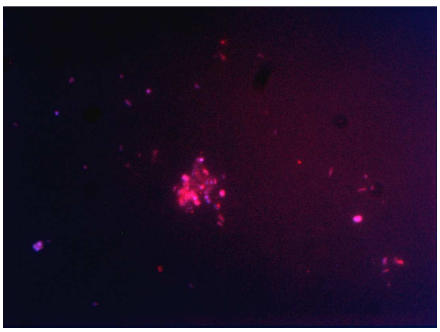


Figure 4.5. FISH micrographs for CAS reactor. Denitrifying bacteria are marked in pink by superposition of blue (DAPI) and red (Pae997).

The AOB population found in HAS reactor belonged to halophilic and halotolerant *Nitrosomonas spp*., obtaining more positive results with probes NEU653 (Figure 4.6-A). Moreover, probes for identification of heterotrophic and denitrifying bacteria, *Pseudomonas spp*. gave also positive results (Figure 4.6-B). However, no NOB population belonged to *Nitrobacter* (NIT3) was detercted.

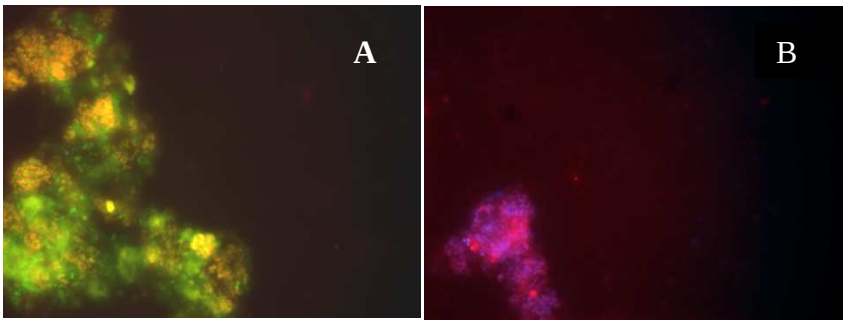


Figure 4.6. FISH micrographs corresponding to HAS reactor. A) Ammonia-oxidizing bacteria are marked in orange by superposition of green (NEU653) and red (BET42a). B) DNA is marked in blue by DAPI and *Pseudomonas spp.* bacteria are marked in pink by superposition of blue (DAPI) and red (Pae997).

4.4.3. Removal of PPCPs from the aqueous phase in CAS and HAS reactor

Considering the average concentration profiles of PPCPs in the aqueous phase from the influent and effluent in both reactors, different removal behaviours were observed.

Anti-inflammatory compounds

High removal efficiencies were observed in both reactors with respect to IBP. CAS system showed a high elimination during the first two sampling campaigns (85-96%) being its removal almost complete afterwards (Figure 4.7). Also in HAS system, a complete removal of IBP (99%) was observed from the beginning of the operation (Figure 8). IBP is a non-steroidal anti-inflammatory drug (Joss et al., 2006; Ternes et al., 2004; Jones et al., 2006) with a low affinity for solids and organic phases and a relatively high biodegradation kinetics coefficient (9-35 L g SSV^{-1} d^{-1}). Due to these characteristics the main removal mechanisms expected is biodegradation, as reported in conventional sewage treatment plants (Suárez et al., 2010; Jones et al., 2007) and also in reactors with a biofilm configuration (Zwiener et al., 2003; Winkler et al., 2001).

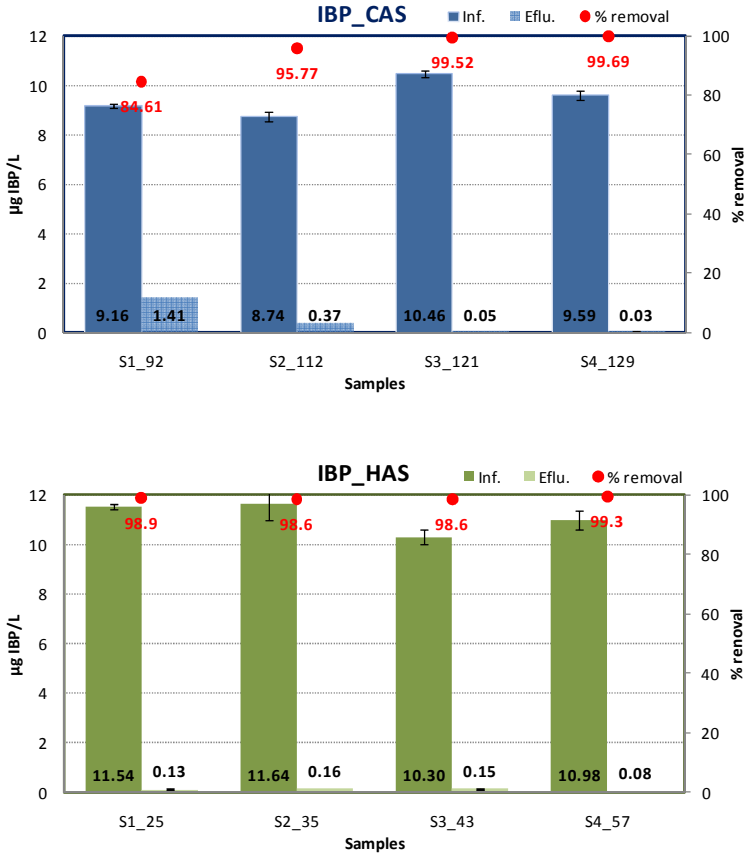


Figure 4.7. Concentration of IBP in the inlet and outlet of CAS and HAS system.

NPX exhibited a slightly different behaviour as its removal efficiency showed a gradual increase from 15 to 88% during the entire operation in CAS system (Figure 4.8); this enhancement was attributed to a possible adaptation period to NPX. Acclimation period is possible to occur due to development of a diversified biota, including slowly-growing bacteria such as nitrifying bacteria which are able to degrade certain pollutants after a period of time (Suárez et al., 2010; Batt et al. 2006; Pérez et al., 2005). However, the behaviour of NPX in HAS system was different because a high removal efficiency >95% was reported since the beginning of the operation (Figure 4.8). Similarly to IBP, the main removal mechanism considered for NPX is biodegradation.

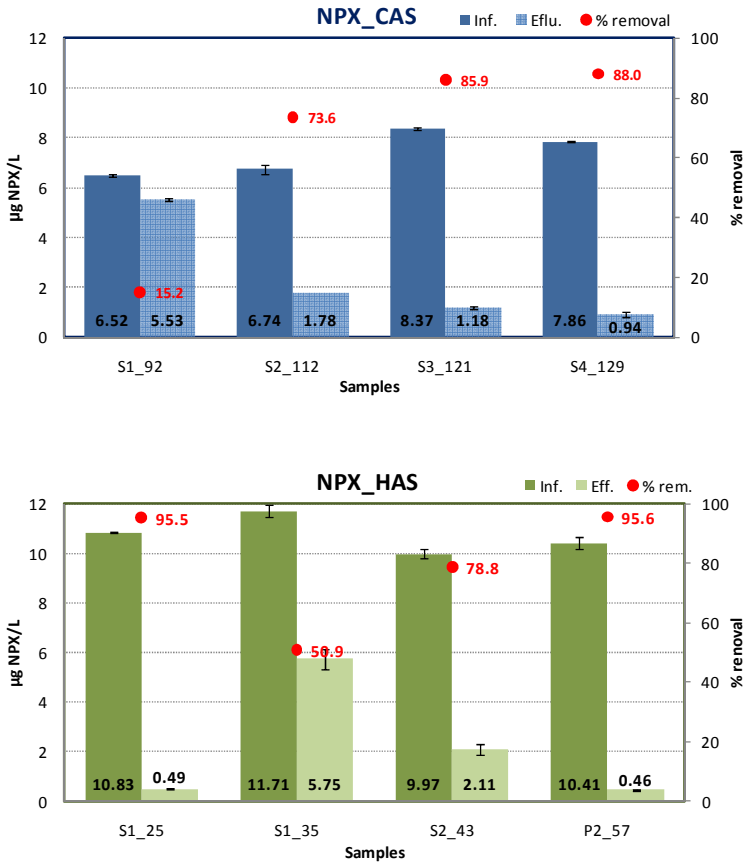


Figure 4.8. Concentration of NPX in the inlet and outlet of CAS and HAS system.

DCF is also an anti-inflammatory acidic compound, although with higher lipophilicity (log K_{ow} 4.5) and affinity to solids (log K_d 1.2). Removal efficiency in CAS system was intermediate and almost constant in all operation, reaching values between 70-75%. A contradictory behaviour in HAS system was observed with global removal efficiency varying in a range from 1 to 55% (Figure 4.9). Literature data on removal of DCF in activated sludge systems are still very contradictory. However, the DCF removal efficiency obtained in CAS system was similar to that reported by the other studies carried out under aerobic conditions (Suárez et al., 2010; Zwiener et al., 2003).

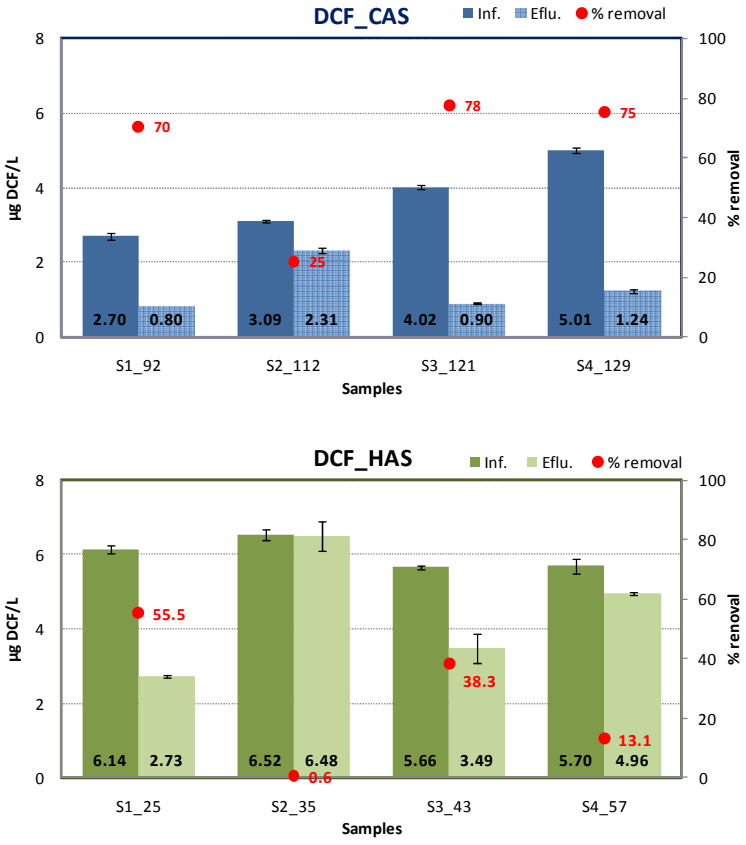


Figure 4.9. Concentration of DCF in the inlet and outlet of CAS and HAS system.

Carbamazepine and Diazepam

CBZ was not removed at all in any system (Figure 4.10), whereas DZP has not been significantly transformed (<20 %) (Figure 4.11). The resistance to biological transformation of both compounds was previously observed by other researchers (Suárez et al., 2010; Radjenovic et al., 2009; Castiglioni et al., 2006; Joss et al., 2006).

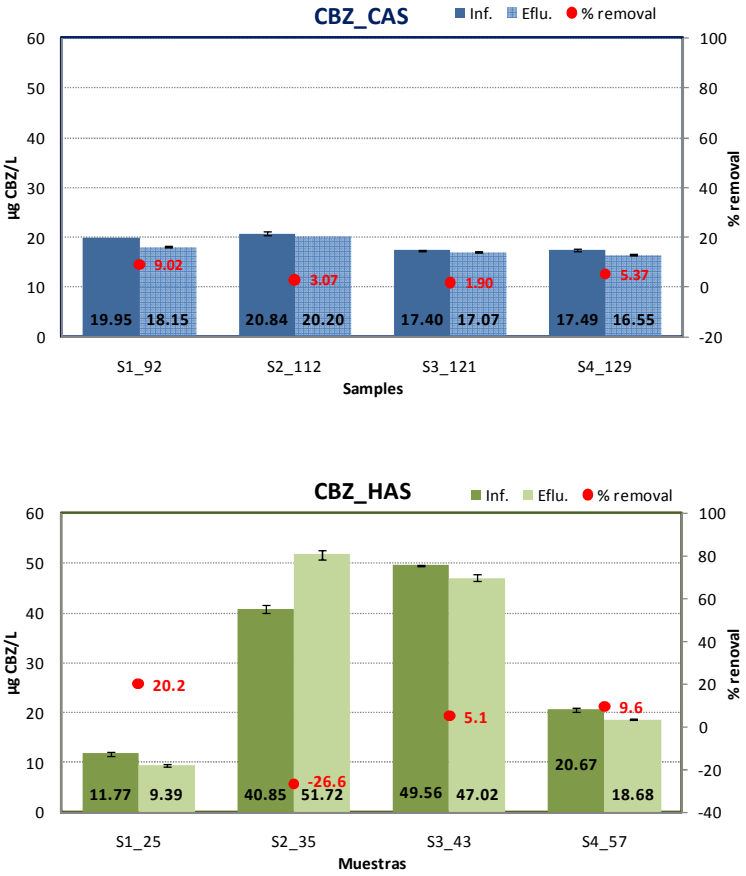


Figure 4.10. Concentration of CBZ in the inlet and outlet of CAS and HAS system.

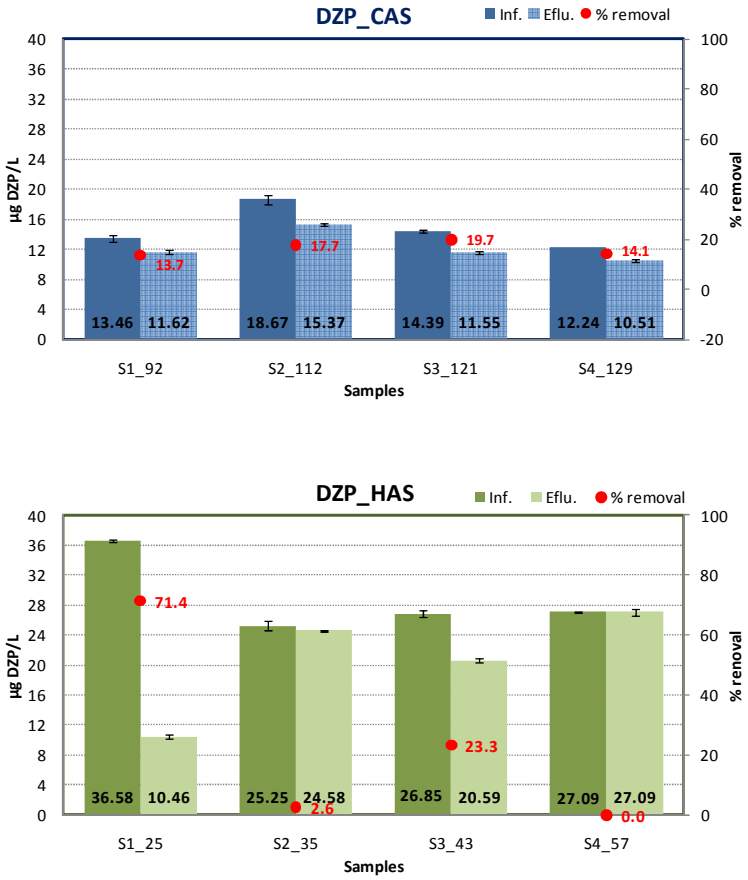


Figure 4.11. Concentration of DZP in the inlet and outlet of CAS and HAS system.

Musk fragrances

GLX, TON and CEL in CAS were also completely removed, reaching ranges of 96-99%, 95-99% and 97-99%, respectively. However, in HAS system efficiency was slightly lower and values from 90-96% for GLX, 88-97% for TON and 92-98% for CEL were achieved during the entire time of operation (Figure 4.12-4.14). The results obtained in this study are comparable with other reports which obtained similar results for these compounds in a pilot (Serrano et al., 2010; Suárez et al., 2010) and full-scale sewage treatment plant (Carballa et al., 2004).

Polycyclic musk fragrances belong to the substances with the lowest solubility in water (<2 mg L^{-1}), being this characteristic reinforced by their strong lipophilic character indicated by the high log K_{ow} values (Table 2).

Both facts explain the affinity to the solid phase which is reflected by their high log K_d values (3.3-3.9). Therefore, according to their physico-chemical characteristics, the possible mechanisms associated to musk elimination are sorption onto sludge (Joss et al., 2005; Kupper et al., 2006). Also, a possible significant sorption on Kaldnes supports could took place in HAS system.

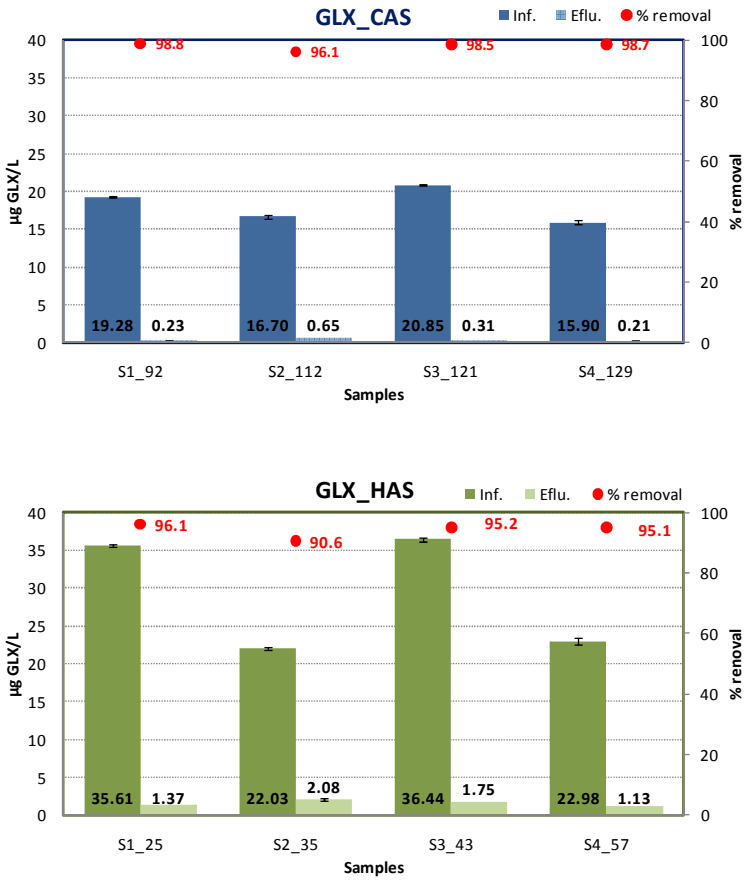


Figure 4.12. Concentration of GLX in the inlet and outlet of CAS and HAS system.

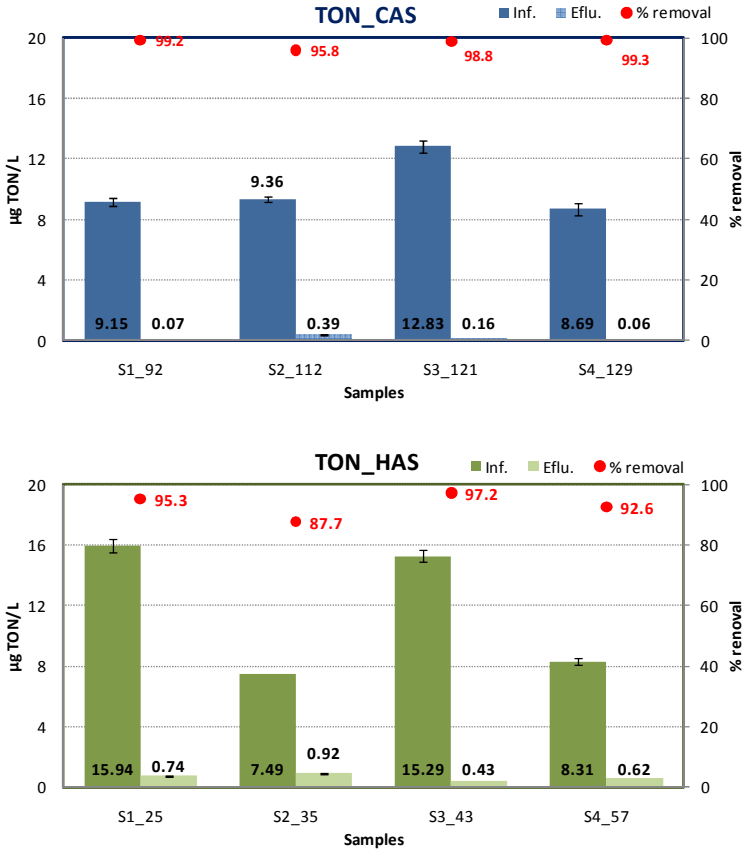
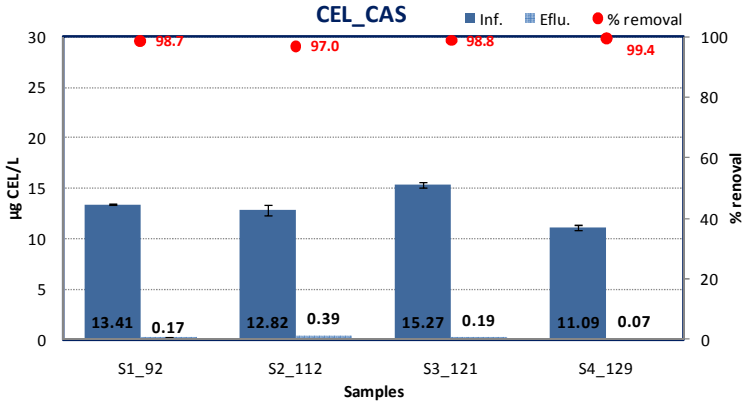


Figure 4.13. Concentration of TON in the inlet and outlet of CAS and HAS system.



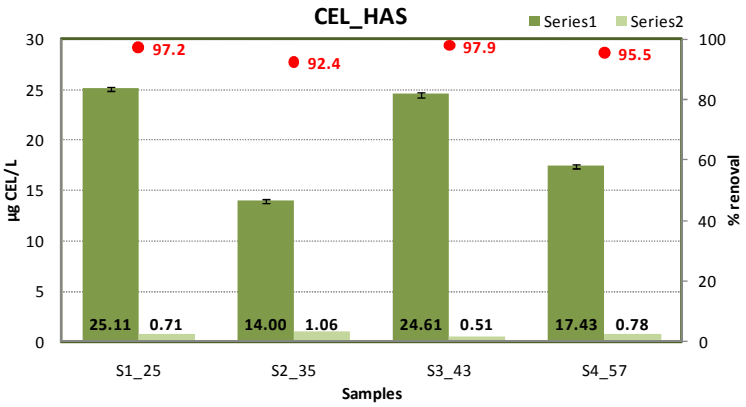


Figure 4.14. Concentration of CEL in the inlet and outlet of CAS and HAS system.

4.5. CONCLUSIONS

A Hybrid Aerobic System (HAS) combining both fixed and suspended biomass and a Conventional Activated Sludge (CAS) reactor were considered to evaluate the PPCPs removal. Both systems were operated for 60 d. The results obtained in both reactors were very similar and therefore, no significant differences in terms of PPCPs removal were observed. HAS reactor showed higher capacity to remove nitrogen ($N\text{-}NH_4^+$) that was confirmed by the detection of some groups of bacteria such as ammonia oxidizers and denitrifiers, whereas in CAS system removal of nitrogen was slightly lower. The slow biofilm growth observed on Kaldnes rings may be the cause that no significant differences were observed between the reactors.

According to the evaluation period in both reactors, the following tendencies can be derived in terms of PPCPs removal.

- Compounds with recalcitrant behaviour:
  - no removal (<10%), eg the case of carbamazepine in both reactors (HAS, CAS);
  - removal <20%, eg diazepam in both reactors.
- Compounds with high removals (>90%): eg musk fragrances (tonalide, celestolide and galaxolide).
- Compounds with high removals in HAS reactor: naproxen and ibuprofen with removal efficiencies >96%.
- Compound with high removals in CAS reactor: diclofenac with removal efficiencies between 70 and 78%.

As a conclusion, only a slight improvement of the removal of IBP and NPX was observed in the HAS system, while for the rest of compounds no conclusive trends are obtained. As a next step, it would be useful to assess the interest of hybrid systems with well-developed biofilm for PPCPs removal.

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Chapter 5

Influence of the employment of adsorption and coprecipitation agents for the removal of PPCPs in Conventional Activated Sludge (CAS) systems[1]

ABSTRACT.- Three activated sludge reactors were operated to improve the removal of organic micropollutants such as Pharmaceutical and Personal Care Products (PPCPs). Reactor 1 (R1) was operated as a Conventional Activated Sludge reactor (CAS), Reactor 2 (R2) consisted of a CAS unit that was continuously fed with $FeCl_3$ whereas granular activated carbon (GAC) was fed directly into the mixed liquor of Reactor 3 (R3) in order to attain concentrations in the range 0.1-1 g L^{-1}. PPCPs removal efficiencies varied depending on the compound present in each reactor during the entire 220 days of operation. Some substances showed the same behaviour in all reactors, such as the acidic pharmaceuticals naproxen and ibuprofen, which were almost completely removed (>90%). More lipophilic organic substances, like musk fragrances, were about 90% removed after 40 days of operation in all of the reactors. The main difference between the three reactors was obtained in R3 when the GAC concentrations in the aeration tank were around 0.5-1 g L^{-1}. Under these conditions, the more recalcitrant compounds like diazepam and carbamazepine could be removed by up to 40%, and diclofenac up to 85%. Adsorption isotherms for PPCPs were obtained with activated carbon, and the results were successfully fitted to the Freundlinch equation. The more recalcitrant compounds (carbamazepine, diazepam and diclofenac) had the highest adsorption capacities onto GAC, which is consistent with the behaviour observed in R3 and helps to identify the removal mechanism (adsorption for these compounds, whereas absorption for fragrances).

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OUTLINE

5.1. INTRODUCTION

Urban sewage has been recognized as one of the main sources of Pharmaceutical and Personal Care Products (PPCPs) in the environment. PPCPs are not completely metabolized in the body, so they are excreted in urine or faeces. Other significant sources of these substances include the effluents from hospitals and landfills (Suarez et al., 2008). Since PPCPs are released into the environment by different ways, Sewage Treatment Plants (STPs) receive wastewaters that contain a wide variety of these substances in concentrations that are commonly found between micrograms and nanograms per litre. The concentrations of PPCPs in the effluent of a STP will depend on the effectiveness of the technology used to remove these micropollutants, but these effluents are commonly considered as important sources of this type of pollution. Moreover, discharge of excess sludge on agricultural land has been reported as a potential risk due to their significant content of the more hydrophobic PPCPs (Kimura et al., 2007; Yu et al., 2008; Suárez et al., 2008).

Some modifications to conventional activated sludge treatment plants have been suggested to improve the removal of conventional pollutants; such modifications may include the use of coagulants or sorption materials (Suárez et al., 2008). Currently, there are processes that use the addition of metallic salts to improve phosphorus removal, such as aluminium, calcium and iron salts. Carballa et al. (2005) reported removal percentages of 50-70% for musk fragrances and acidic compounds when using different coagulants during the primary treatment stages; ferric chloride produced the best results. Similarly, Suarez et al. (2009) assayed the removal of 13 PPCPs during the primary treatment of hospital effluents using aluminium and iron salts, obtaining removal percentages around 90% for musk fragrances (galaxolide, tonalide and celestolide) and 30-60% for acidic compounds (ibuprofen, naproxen and diclofenac).

Another alternative to enhance PPCPs removal is the use of sorption agents, such as Powdered Activated Carbon (PAC) or Granular Activated Carbon (GAC). Activated carbon appears to be an attractive alternative since it exhibits a large adsorption capacity for a wide number of pollutants. With ultra pure water, Ternes et al. (2004) studied the removal of four PPCPs (benzafibrate, clofibric acid, diclofenac and carbamazepine) using GAC, confirming that all of them can be efficiently removed under those conditions. With activated sludge processes, Ng and Stenstrom (1987)

assayed that the use of 0.5-4 g L^{-1} of PAC may enhance nitrification by 75-97%, whereas other authors observed an improvement of organic matter removal as well as a significant decrease of toxicity caused by certain inhibitors on the nitrification process (Widjaja et al., 2004). Finally, Vidic and Suidan (1991) reported that micropollutant adsorption is more efficient if the GAC is added directly into the aeration system.

5.2. OBJECTIVES

The aim of this work is to evaluate different modifications to the Conventional Activated Sludge (CAS) process, such as adding coagulants ($FeCl_3$) or GAC directly into the aeration tank, in order to enhance the removal of PPCPs from sewage.

5.3. MATERIALS AND METHODS

5.3.1. Granular Activated Carbon

The experiments were conducted with GAC (4.96% ash, 2.45 ± 0.11 g cc^{-1} real density, 0.76 g cc^{-1} apparent density, 1003.8 m^2 g^{-1} surface area, 68.9% porosity, 0.02 μm pore-size) obtained from MERCK, Spain. Only the 1 mm mesh fraction of carbon was used after being washed three times with ultrapure water, dried at 105 °C overnight and finally storaged in a desiccator.

5.3.2. Adsorption experiments

Batch adsorption studies were performed with 250 mL flasks shaken at 130 rpm and 25 °C during 48 h to achieve adsorption equilibrium. All experiments were carried out by contacting the corresponding amount of adsorbent (0.01 g GAC L^{-1}) with 600 mL of a buffered PPCPs solution (5-75 μg L^{-1}) in aluminum flasks. After shaking, samples from the liquid were filtered to remove any suspended carbon particles and PPCPs content was analysed. Freundlich model was used to fit the experimental data.

5.3.3. Analytical methods

Samples were regularly taken from the influent and effluent of each reactor. COD, TSS, VSS, N-NH_4, N-NO_3 and P-PO_4 were determined according to standard methods (APHA, 1999). Additionally, the soluble concentrations of the PPCPs in the influent and effluent were analysed. These concentrations were determined after solid phase extraction (SPE) of 250 mL samples using 60 mg OASIS HLB Cartridges (Waters, Milford, MA, USA). The samples were

filtered and the pH was adjusted to 2.5 prior to the SPE process (Rodríguez et al., 2003).

5.3.4. Operation of the activated sludge reactors

Our experiments employed three laboratory-scale activated sludge plants (Figure 5.1), namely reactors R1, R2 and R3. Each reactor consisted of a 2 L aerated vessel coupled with a 1 L clarifier. R1 was a control reactor, i.e. a conventional activated sludge system treating synthetic sewage. On the other hand, R2 was continuously fed with $FeCl_3$, a common metallic salt used for phosphate removal, and R3 was supplemented with several discrete additions of GAC in order to achieve different concentrations of activated carbon (0.1 to 1 g L^{-1}) in the aeration tank.

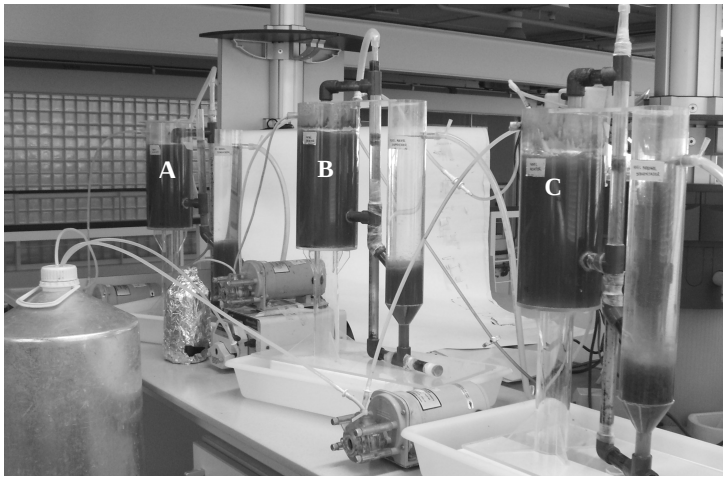

Figure 5.1. Activated sludge bench scale pilot plant. A) Control reactor, R1; B) Reactor with addition of ferric salts as coagulant, R2; and C) Reactor with addition of different concentrations of GAC, R3.

The three reactors were inoculated with sludge (4160 mg TSS L^{-1} and 3160 mg VSS L^{-1}) obtained from a full-scale municipal waste water treatment plant located in NW Spain. The reactors were fed with a synthetic municipal wastewater feeding (500 mg L^{-1} COD, 40 mg L^{-1} N NH_4^+, 8 mg L^{-1} P-PO_4^{3-}, 200 mg L^{-1} $NaHCO_3$ and trace minerals). An inlet flow rate of 2 L d^{-1} was applied, which corresponds to a hydraulic residence time of 1 d.

Once a steady-state was achieved, PPCPs were spiked into the influent in order to ensure concentrations of 10-40 µg L^{-1} in all reactors, such as those

commonly found in urban wastewaters. 20 μg L^{-1} of carbamazepine (CBZ) and diazepam (DZP); 40 μg L^{-1} of galaxolide (GLX), tonalide (TON) and celestolide (CEL); 10 μg L^{-1} of ibuprofen (IBP), naproxen (NPX) and diclofenac (DCF).

The metallic salt was added continuously to R2 as a solution of $FeCl_3 \cdot 6H_2O$. Taking into account that the phosphorus fed to each reactor in the synthetic wastewater was 16 mg d^{-1}, the coagulant solution flow was adjusted to 150 mg d^{-1}.

5.4. RESULTS AND DISCUSSION

5.4.1. Adsorption experiments

Adsorption isotherms for PPCPs on GAC at 25 °C in Milli-Q water were obtained. Experimental data were used fitted to the Freundlich equation: $\log q = \log K_f + (1/n) \log C_e$; where q is the adsorption capacity (μg PPCPs adsorbed/g activated carbon), Ce the final equilibrium concentration of PPCPs in the liquid phase (μg L^{-1}), K_f and 1/n are the Freundlich parameters. Table 5.1 summarizes the results obtained for K_f and 1/n for the selected PPCPs.

Table 5.1. Freundlich parameters for adsorption isotherms obtained with GAC for selected PPCPs.

Compuesto	**K_f (μg g^{-1}) (L $\mu g^{-1})^{1/n}$**	**1/n**	**R^2**
Ibuprofen	72	0.87	0.99
Naproxen	107	0.87	0.99
Diclofenac	170	0.85	0.88
Carbamazepine	565	0.25	0.87
Diazepam	110	0.81	0.99
Galaxolide	22	0.83	0.79
Tonalide	17	1.20	0.90
Celestolide	41	0.83	0.93

The constant K_f characterizes the adsorption capacity whereas 1/n is related to the intensity of the adsorption force between the activated carbon surface and the adsorbate. The values of K_f calculated for initial PPCPs concentrations around 5-75 μg L^{-1}, were in the 17-565 (μg g^{-1}) (L $\mu g^{-1})^{1/n}$. It was found that CBZ, DZP, DCF and NPX, had higher adsorbability onto GAC at neutral pH than the other compounds such as musk fragrances.

The results obtained are in accordance with those previously reported by Yu et al. (2008), who evaluated the adsorption of two pharmaceutical compounds (CBZ and NPX) and one endocrine disrupting compound (nonylphenol). They found that the removals of target compounds were contrary to expectations based on their hidrophobicity: CBZ exhibited the highest adsorption among the three compounds (log Kow 2.45) whereas the nonyphenol (log Kow 5.8) was most poorly adsorbed. Also, Ternes et al. (2002) reported the highest K_f values for CBZ in comparison with other three pharmaceuticals, in batch assays carried out with pulverized GAC working both with Milli-Q water and groundwater.

Little differences were noted among the isotherms obtained for the three musk fragrances, all of them showing lower adsorption capacity onto GAC. This fact can be explained by their physico-chemical properties, since they are not ionised, have a low solubility in water and a remarkable lipophilic behaviour, which makes them very likely to be absorbed into the organic fraction of the suspended solids, but not prone to interact by adsorption.

5.4.2. Operation of the activated sludge reactors

The start-up period lasted 41 days (Period 0), during which all of the reactors were operating as conventional CAS units. From day 42 onwards, the feeding solution was spiked with the selected PPCPs, and during the following 33 days (Period 1) the performance of the three reactors was monitored to determine the effects of this addition. In the following periods the conditions were different for each reactor: the operation of R1 was not changed since this was the control unit; $FeCl_3$ was continuously added to the aeration tank of R2 in order to maintain a concentration of 15 mg L^{-1} in the influent; and R3 received a single addition of GAC on the first day of each period, achieving final concentrations of 0.1 g L^{-1} (Period 2), 0.5 g L^{-1} (Period 3) and 1 g L^{-1} (Period 4) in the aeration tank. During the entire operation, the temperature and pH were not controlled but monitored, staying in the range of 18-24 °C and 8.2-8.9, respectively. The oxygen concentration was maintained above of 8 mg L^{-1} during the entire operation for all of the reactors (216 days).

Once all of the reactors were operated at steady conditions and due to the absence of purges, the biomass content increased in all reactors. The biomass increased the most in reactor R2 due to a continuous supply of the coagulant and the consequent accumulation of precipitates. At the end of P3, the TSS biomass concentrations reached 5 g L^{-1} in R2 and R3 with a remarkably

higher concentration of inert material in R2. Maximum biomass values were attained during P4; of the highest value was in R3 due to the presence of 1 g L^{-1} of GAC. Due to these high values, during the last period (P4) purges were performed in order to maintain the level of TSS to 5-6 g L^{-1} in all reactors.

The organic matter removal efficiencies were around 95% in terms of the soluble Chemical Oxygen Demand (COD) level, which was achieved at the beginning of P1. The same behaviour was observed for the ammonia nitrogen removal; there were no significant differences within periods P1-P4. R1 and R3 achieved about 20% removal of phosphorus. Phosphorus removal was substantially higher in R2 due to the addition of $FeCl_3$, reaching values of 60-70%. Previous studies have reported the benefits of continuously feeding the coagulant to the aeration tank in MBR systems; the benefits include not only an enhanced phosphorus removal, but also a reduction of fouling in membranes (Song et al., 2008). However, the presence of GAC in R3 had no positive effect on the removal of phosphorus, as the removal percentage was in the same range as R1 (10-20%). The addition of pharmaceuticals during P1 did not cause any significant disturbance on the biological process of any of the reactors, as was expected from previous reports (Suárez et al., 2005; Reif et al., 2008).

5.4.3. PPCPs removal

Ibuprofen and Naproxen

IBP was highly degraded during the first weeks of operation (around 85-95% removal in all reactor systems) and it was totally removed (99%) from all systems by the end of Period 2 (from day 112 onwards). NPX exhibited a similar pattern, although it was more resistant to complete removal: only 90% was achieved after periods P1 and P2 (Figure 5.2). As observed with ibuprofen, no difference was observed within the three reactors, which indicates the low affinity of these compounds for the metal precipitates of R2 or the activated carbon present in R3.

Both compounds are acidic, with a moderate solubility in water and a low affinity to solids or organic phase. At the circumneutral conditions existing in wastewater treatment plants, these substances are mainly in the aqueous phase, which is enhanced by their hydrophilic nature. The higher biodegradation kinetic coefficient (9–35 L g^{-1} SS d^{-1}) explains that ibuprofen is faster degraded. However, both compounds required an initial period (around 100-130 days) in order to achieve their maximum removal

efficiencies. This time period might be correlated with the increase of the biomass, or more precisely, with the population of nitrifiers, since the highest value of ammonia removal was also achieved at the end of Period 2. Similar results were also obtained by Suárez et al. (2005). Results for the behaviour of IBP have been confirmed by Kimura et al. (2007), Radjenovic et al. (2007) and Reif et al. (2008), who reported removal efficiencies around of 90%. For NPX, Radjenovic et al. (2007) reported removal efficiencies between 85-99%, whereas lower removal capabilities were found by Suárez et al. (2005) and Reif et al. (2008), 68% and 84%, respectively. As a result, it can be concluded that the main removal mechanism for both compounds is biodegradation, which can be enhanced with the development of biomass rich in nitrifiers.

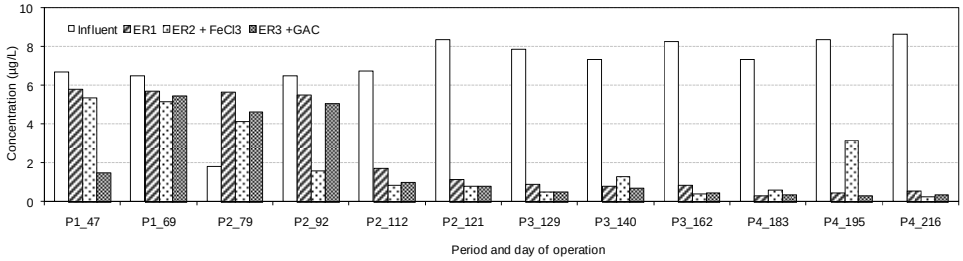

Figure 5.2. Concentrations of NPX in the inlet and outlet of R1, R2 (with $FeCl_3$) and R3 (with GAC).

Diclofenac

DCF is an acidic compound like ibuprofen and naproxen. However, it is less soluble in water and has a medium character between lipophilicity and hydrophilicity, as indicated by its K_{ow} and K_d values (Suárez et al., 2008). In addition, it is more recalcitrant to biological degradation and more problematic to be determined, as shown by the frequent problems caused during analytical determination. As a result, removal data are relatively varied (Figure 5.3), which makes them more difficult to analyse. However, statistical analysis shows that the presence of GAC gave better results, mainly during P3 where the removal was 85%, compared with 74% (R1) and 70% (R2). This is concordant with the high value obtained for the Freundlich parameter K_f in comparison with the other PPCPs (Table 5.1). In fact, the addition of the coagulant did not enhance its removal.

Recent results show differences in DCF removal efficiencies as a function of the technology used. The addition of a coagulant in a coagulation-flocculation pilot plant improved the removal efficiencies up to 50-60% (Suárez et al.

2009). Ternes et al. (1998) reported a relatively high removal efficiency (69%) in a wastewater treatment plant. On the other hand, Kimura et al. (2007) and Radjenovic et al. (2007) found similar conclusions when comparing CAS and MBR units: 40-50% removal efficiencies were obtained in CAS systems, whereas 80-87% removal efficiencies were reported for MBR systems. These differences are justified according to the higher SRT commonly found in MBR systems.

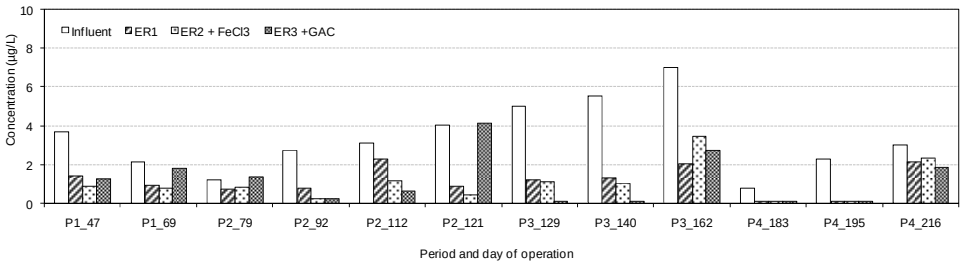

Figure 5.3. Concentrations of DCF in the inlet and outlet of R1, R2 (with $FeCl_3$) and R3 (with GAC).

Galaxolide, Tonalide and Celestolide

The musk fragrances exhibited the same behaviour in all units, as shown for TON in Figure 5.4. High removal efficiencies were observed by the end of P1 (with average values ranging 71-92%), which further increased in the following sampling campaigns, with values ranging from 95 to 99%. These results are in accordance with those previously reported by Carballa et al. (2004), who obtained removal efficiencies around 70-90% for these compounds in a full-scale sewage treatment plant.

In contrast with the acidic compounds previously discussed, these substances are of lipophilic nature (log K_{ow} 5.4-6.6) and their main removal mechanism in STPs has been reported to be sorption onto sludge or, more specifically, removal by absorption in the lipophilic cell membrane of the microorganisms and the lipid fractions of the sludge. Since interaction by adsorption was not so important, as indicated by values of K_f (Table 5.1), absorption was the main removal mechanism in our reactors. This evidence is also supported by the correlation observed between the increase in TSS and the enhancement of the removal efficiencies after the first weeks of operation.

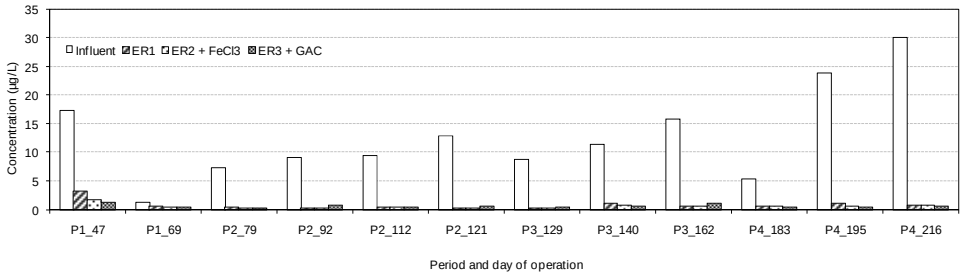

Figure 5.4. Concentrations of TON in the inlet and outlet of R1, R2 (with $FeCl_3$) and R3 (with GAC).

Carbamazepine and Diazepam

CBZ and DZP are both neutral compounds which have been reported to have a low level of interaction with solids and a strong recalcitrant character, particularly for CBZ as deduced from their low K_{biol} values (<0.01 L g^{-1} SS d^{-1}). Figure 5.5 shows the concentrations of CBZ in the reactors. This compound was removed by up to 43% in R3 during P4, when the GAC was a concentration of 1 g L^{-1}. By contrast, no significant removal was reported in the other two reactors (<12%).

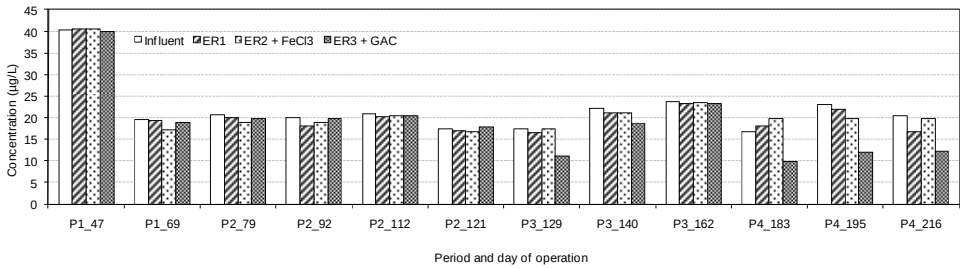

Figure 5.5. Concentrations of CBZ in the inlet and outlet of R1, R2 (with $FeCl_3$) and R3 (with GAC).

Figure 5.6 shows the results for DZP that are similar to the results for CBZ: there was no significant difference between R1 and R2 (with removal efficiencies around 10-25%), while the best results were achieved in R3 where the GAC concentration was either 0.5 or 1 g L^{-1}, leading to removal efficiencies of 35% and 32%, respectively.

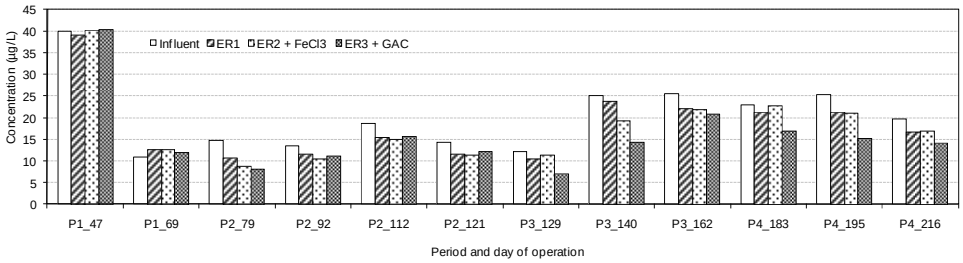

Figure 5.6. Concentrations of DZP in inlet and outlet of R1, R2 (with $FeCl_3$) and R3 (with GAC).

Previous studies have shown that CBZ can be persistent in wastewater treatment processes (Clara et al., 2004; Suárez et al., 2005; Castiglioni et al., 2006; Radjenovic et al., 2007; Reif et al., 2008). Stackelberg et al. (2007) confirmed this behaviour, obtaining removal efficiencies less than 15% using $FeCl_3$ as coagulant. Similar low removal efficiencies were reported for DZP (Suárez et al., 2005; Joss et al., 2006; Reif et al., 2008). Therefore, since neither biodegradation nor absorption seem to be significant as removal processes for these substances; their higher affinity to activated carbon could be used to improve their elimination by adsorption, even in CAS aeration tanks as evidenced in this work.

5.5. CONCLUSIONS

The employment of a coagulant (ferric chloride) and an adsorbent (activated carbon) was studied in three lab-scale activated sludge reactors in order to evaluate the potential enhancement of PPCPs removal. While the continuous addition of a coagulant in the aerated tank showed no significant effect on the removal of micropollutants (reactor R2), the addition of GAC directly into the aerated tank caused the enhancement of the more recalcitrant pharmaceuticals micropollutants studied, such as carbamazepine, diazepam and diclofenac (reactor R3). On the other hand, the results obtained from the determination of adsorption isotherms showed that these recalcitrant substances have higher affinity to activated carbon than acidic pharmaceuticals (ibuprofen, naproxen) and musk fragrances (galaxolide, tonalide and celestolide). All of these evidences indicate that during the operation of three reactors acidic micropollutants such as ibuprofen or naproxen are mainly removed by biodegradation; musk fragrances are mainly removed by absorption into the lipophilic fraction of the sludges; whereas carbamazepine and diazepam are only partially removed by

adsorption onto the activated carbon. Thus, the use of activated carbon as GAC may be an efficient tool to increase the removal of carbamazepine, diazepam and diclofenac.

5.6. REFERENCES

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Chapter 6

Removal of persistent pharmaceutical micropollutants from sewage by addition of PAC in a sequential membrane bioreactor

ABSTRACT.- The performance of a membrane bioreactor operating in a sequential mode (SMBR) using an external flat-plate membrane was investigated. After 200 days of operation, a single addition of 1 g L^{-1} Powdered Activated Carbon (PAC) was added directly into the mixed liquor in order to enhance the simultaneous removal of nutrients and pharmaceutical micropollutants from synthetic urban wastewater. Throughout the entire operation (288 days), Chemical Oxygen Demand (COD) removal efficiencies were up to 95%, ammonium nitrogen removal was maintained over 70-80%, whereas phosphorus removal achieved only high values (around 80%) after PAC addition. During the operation of the SMBR without PAC addition, micropollutants which exerted a more recalcitrant behaviour were carbamazepine, diazepam, diclofenac and trimethoprim, with no significant removal. On the other hand, moderate removals (42-64%) were observed for naproxen and erythromycin, whereas ibuprofen, roxithromycin and fluoxetine were removed in the range of 71-97%. The addition of PAC into the aeration tank was a successful tool to improve the removal of the more recalcitrant compounds up to 85%. The highest removal with PAC was observed for carbamazepine, trimethoprim as well as for roxithromycin, erythromycin and fluoxetine. All of these five compounds have amine groups and pKa slightly above neutrality (7-10), thus the interaction between PAC and the positively charged amino groups might be the cause of their comparatively better results. Microbial ecology present in the biomass showed a higher abundance of Accumulibacter phosphatis as well as the ammonium oxidizing bacteria belonging to the genus Nitrosomonas after PAC addition.

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OUTLINE

6.1. INTRODUCTION

Due to the incomplete removal of the most recalcitrant organic micropollutants detected in urban wastewaters during conventional treatment processes (Radjenovic et al., 2007; Castiglioni et al., 2006; Lindqvist et al., 2005; Carballa et al., 2004) it is necessary to develop new treatment concepts to adequately address this problem. Among the different studies carried out in the last decade, different strategies appear of interest such as the implementation of anoxic-aerobic processes for nitrogen removal; the use of Membrane Biological Reactors (MBRs); or the feeding of additives such as adsorbents directly into the aeration tank of activated sludge processes.

Different works have reported that alternating anoxic-aerobic processes, as existing in modern Sewage Treatment Plants (STPs) designed for biological nitrogen removal, can be useful to improve the degradation of certain micropollutants (Suarez et al., 2010; Andersen et al., 2003; Zwiener et al., 2000). Among others, Sequential Batch Reactors (SBRs) constitute an interesting alternative in which anoxic and aerobic conditions can be easily combined and controlled in a single stage. Some of their main features are their low footprint (secondary settler not needed) as well as their ability to face shock loads and the operation even under the presence of inhibitory substances (Andreottola et al., 2001; Strous et al., 1998; Sirianuntapiboon and Ungkaprasatcha, 2007).

Membrane Biological Reactors (MBR) have gained significant popularity in (STPs) and are nowadays considered as a powerful (and expensive) technology able to produce higher quality effluents in terms of conventional pollutants, which can be appropriate for direct discharge, further postreatment or even reuse purposes. However, since membrane filtration does not enhance the elimination of most micropollutants by means of a size-exclusion mechanism it is still not clear if these systems may effectively enhance the removal of organic micropollutants (Reif et al., 2008, Radjenovic et al., 2007; Clara et al., 2005).

Powdered and Granular Activated Carbon (PAC and GAC) have been commonly used for sorption of organic micropollutants like pesticides or taste and odor compounds (Ternes and Joss, 2006). Previous studies carried out in samples with no or extremely low organic matter content (ultra-pure water or samples from Drinking Water Plants, respectively) reported successful removal of a wide number of complex and recalcitrant

micropollutants including Pharmaceutical and Personal Care Products (PPCPs) and Endocrine Disrupting Compounds (EDCs) commonly found in urban wastewaters (Yu et al., 2008; Snyder et al, 2007; Westerhoff et al., 2005). However, activated carbon addition is not common in STPs. With activated sludge processes, Ng & Stenstrom (1987) showed that the use of 0.5–4 g L^{-1} of PAC may enhance nitrification rates by 75–97%, whereas other authors observed an improvement of organic matter removal as well as a significant decrease of toxicity caused by certain inhibitors on the nitrification process (Widjaja et al., 2004). In fact, activated carbon is a suitable support for bacterial attachment, being possible in this way to enhance the retention of the more slowly growing bacteria, such as nitrifiers (Thuy and Visvanathan, 2006; Aktas and Cecen, 2001). Previous studies carried out by our group (Serrano et al., 2010) showed that a GAC addition of 0.5-1 g L^{-1} directly into the aeration tank of an activated sludge reactor can be a useful tool to increase the removal of the recalcitrant PPCPs carbamazepine, diazepam and diclofenac. Moreover, recent works have shown that activated carbon can be useful to minimise fouling problems in MBRs. In this way, the use of PAC concentrations of 0.5-3 g L^{-1} inside the aeration tank of a MBR have been used to attain an easier control of membrane fouling (Remy et al., 2009; Munz et al., 2007).

6.2. OBJECTIVES

The aim of this work was to assess the removal of selected pharmaceutical micropollutants contained in synthetic municipal wastewaters using a Sequential Membrane Batch Reactor (SMBR) which comprises an SBR unit coupled with an external MF membrane. Moreover, the addition of PAC directly into the aeration tank will be assessed as a tool to enhance the removal of the more recalcitrant compounds. In this way, the aim was to combine the potential of PAC (sorption of PPCPs and development of a much more diverse microbial ecology) with the advantages of MBRs (higher SRT and sludge concentrations, complete retention of biomass and PAC) in the removal of the target compounds.

6.3. MATERIALS AND METHODS

6.3.1. Experimental Set-up

The experiments were performed in a membrane bioreactor with a sequential mode of operation (SMBR) using an external flat-plate membrane (Figure 6.1).

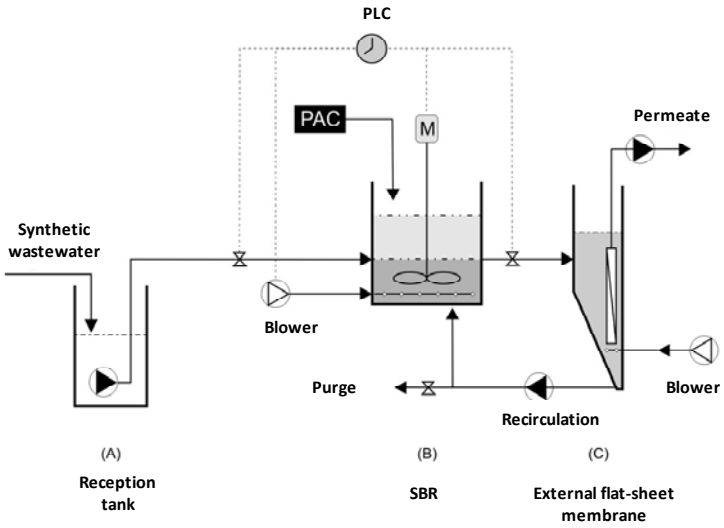


Figure 6.1. Schematic diagram of the membrane bioreactor with a sequential mode of operation (SMBR) using an external flat-plate membrane.

The reactor consisted of two stainless steel units: an SBR, with a working volume of 30 L, and a 18 L vessel which contained the MF flat membrane (Kubota). Recirculation of biomass from the external membrane chamber to the SBR was carried out in order to achieve an external recirculation ratio of 0.23. The membrane has an effective filtration area of 0.1 m^2 and a nominal pore size of 0.4 μm. Air was supplied by distributors placed at the bottom of the SBR and under the membrane module in the second unit. The air flow rate provided oxygen to both units (> 2 mg L^{-1}) and induced shear stress around the membrane to minimise fouling. The membrane was operated intermittently (6.25 min suction and 1.25 min relaxation). A constant flow was continuously maintained. When the Transmembrane Pressure (TMP) exceeded 15 kPa a physical cleaning using pressurized water was performed. The reactor was operated during 288 days divided in three periods. Since no sludge purge was performed, Sludge Retention Time (SRT) increased with time of operation. The start-up period lasted 104 days (P1), until stable conditions were achieved, during which no PPCPs were added to the SMBR. From day 105 onwards (P2), the feeding solution was spiked with the selected PPCPs (Table 6.1) and during the following 96 days the performance of the SMBR was monitored. The selection of the compounds has been based on several criteria previously indicated (Suarez et al., 2008). At the start of P3, which lasted 86 days, the SMBR received a single addition of PAC at a

concentration of 1 g L^{-1}. Commercial QP PAC (code 211237) was purchased from PANREAC and presented the following characteristics: 1.665 g cm^{-3} real density, 0.25 g cm^{-3} apparent density, 328.2 m^2 g^{-1} specific surface area.

Table 6.1. Theoretical concentrations of PPCPs spiked to the SMBR feeding (C_{feed} in µg L^{-1}) and the reported physic-chemical characterisitics: dissociation constant (pKa), octanol-water partition coefficient (log K_{ow}), solid-water distribution coefficient (K_d in kg L^{-1}) and pseudo first-order degradation constant (k_{biol} in L g^{-1}SS d^{-1}).

Compound	C_{feed}	pKa	log K_{ow}	log K_d	k_{biol}[f]
Anti-depressant					
Fluoxetine (FLX)	20	10.1	4.0	0.7[ab]	-
Anti-inflammatories					
Ibuprofen (IBP)	10	4.9-5.2	3.5-4.5	0.9[c]	9-35
Naproxen (NPX)	10	4.2	3.2	1.1[d]	0.4-1.9
Diclofenac (DCF)	10	4.1-4.2	4.5-4.8	1.2[c]	<0.1
Anti-epileptic					
Carbamazepine (CBZ)	20	7	2.3-2.5	0.1[c]	<0.01
Antibiotics					
Trimethoprim (TMP)	10	6.6-7.2	0.9-1.4	2.3[a]	-
Roxithromycin (ROX)	10	9.2	2.1-2.8	2.2[e]	<0.3
Erythromycin (ERY)	10	8.9	2.5-3.0	2.2[a]	0.5-1
Tranquillizer					
Diazepam (DZP)	20	3.3-3.4	2.5-3.0	1.3[c]	<0.03

a)Jones et al. (2002); b) Brooks et al. (2003); c) Ternes et al. (2004); d) Urase and Kikuta (2005); e) Joss et al. (2005) and f) Suárez et al. (2008).

A programmable logic controller was used to operate the reactor using a 6 h cycle with the following phase sequence: filling, 8 min; anoxic reaction, 93 min; aerobic reaction, 236 min; settling, 15 min and effluent withdrawal, 8 min. The SBR unit was operated under anoxic-aerobic conditions, with a Volume Exchange Ratio (VER) of 25% after each cycle. The discharged effluent was fed to the membrane unit, from which a continuous permeate was produced.

The SMBR was seeded with 1.40 g VSS L^{-1} of aerobic sludge obtained from STP located in NW Spain which treats municipal wastewater. No sludge was taken off from the system during the whole experimental periods except for sampling. The reactor was fed with a synthetic mixture with similar characteristics to a high-strength urban wastewater: 1000 mg L^{-1} COD (as $NaCH_3CO_2$), 80 mg L^{-1} N-NH_4 and 8 mg L^{-1} P-PO_4 and a solution of trace elements ($FeCl_3$, H_3BO_3, $CuSO_4$, KI, $ZnSO_4$, $CoCl_2$, $MnCl_2$ at concentrations in

the range of 3-150 μg L^{-1}). An inlet flow rate of 30 L d^{-1} was applied, which corresponds to a hydraulic residence time of 1 d. Samples were taken twice a week from the influent, permeate and mixed liquor in the middle of the aerobic phase of the SMBR in order to analyse conventional parameters. For PPCPs, 4 sampling campaigns were carried out during P2 and 5 during P3, at 2-3 weeks intervals.

6.3.2. Analytical Methods

Samples collected from the reactor were analysed for conventional physical–chemical parameters (COD, TSS, VSS, Sludge Volume Index (SVI), N-NH_4, N-NO_3 and P-PO_4) according to standard methods (APHA, 1999). Additionally, sampling campaigns of the soluble concentrations of PPCPs in the influent and permeate were performed along P2 and P3. The composition of each sample was time-proportional along two cycles. Immediately after collecting 1 L of samples in aluminium bottles, each sample was filtered through a 0.45 μm glass fiber filter. For the analysis of PPCPs sample extraction based on Solid Phase Extraction (SPE) was used as pre-concentration technique prior to their quantitative determination. Liquid or Gas Chromatography coupled with Mass Spectrometry (LC–MS or GC–MS, respectively) was used for the final quantification. Analysis of the soluble content of anti-inflammatory compounds, carbamazepine (CBZ) and diazepam (DZP) was performed following the methodology described in Rodríguez et al. (2003), which consists of adjusting the pH of the samples to 2.5, adding meclofenamic acid and dihydrocarbamazepine as surrogate standards, carrying out a SPE of 250 mL samples using 60 mg OASIS HLB cartridges (Waters, Milford, MA, USA) and a final elution from the cartridge using 3 mL of ethyl acetate. This extract was divided into two fractions: one of them was used for direct determination of CBZ and DZP, while the other one was employed for the analysis of anti-inflammatories as their tertbutyldimethylsilyl derivatives. Finally, GC/MS detection was carried out in a Varian CP 3900 chromatograph (Walnut Creek, CA, USA) equipped with a split–splitless injector and connected to an ion-trap mass spectrometer. The chemical analysis for the determination of fluoxetine (FLX), trimethoprim (TMP), roxithromycin (RXT) and erythromycin (ERY) was performed as published by Vanderford et al. (2003). Solid-Phase Extraction (SPE) was done as described for the previous compounds, although the elution step was performed with a mixture of methanol (1.5 mL) and methyl tert-butyl ether (1.5 mL). Final detection was performed in an LC-MS-MS (analyzed in an Agilent Liquid Chromatograph API 400 GI312A equipped with a binary pump and autosampler HTC-PAL

and the detection was performed with a triple quadruple Mass Spectrometer) in the positive ESI mode.

6.3.3. Morphological Observation and FISH Analysis

Activated sludge was morphologically characterized by phase-contrast microscopy and several staining procedures. Gram, Neisser, Polyhydroxybutyrate (PHB) and sheath stains techniques were performed as described by Jenkins et al. (2004). Filaments were classified according to their morphology, cell inclusions, motility, staining reactions and filament abundance was estimated using the criteria suggested by Kämpfer and Wagner (2002).

Size distribution of the sludge was analysed by laser diffraction technique. The floc evolution, shape and quality of the biomass developed were monitored by stereo-microscope observation at 100 magnifications. Microbial populations were determined by the Fluorescence In Situ Hybridization (FISH) technique. Biomass samples from the reactor were collected, disrupted and fixed, according to the procedure described by Amann et al. (1995), with 4% paraformaldehyde solution. Hybridization was performed at 46°C for 90 minutes adjusting formamide concentrations at different percentages shown in Table 6.2. The used probes for in situ hybridization were 50´ labelled with the fluorochromes FITC and Cy3. Fluorescence signals of disrupted samples were recorded with an acquisition system coupled with an Axioskop 2 epifluorescence microscope (Axioskop 2 plus, Zeiss).

Table 6.2. List of FISH probes used in this work.

Probe	Sequence (5' → 3')	% Formamide	Reference
EUB338I	GTC GCC TCC CGT AGG AGT	20	Amann et al., 1995
ALF1B	CGT TCG YTC TGA GCC AG	20	Manz et al., 1992
BET42a	GCC TTC CCA CTT CGT TT	35	Manz et al., 1992
NEU653	CCC CTC TGC TGC ACT CTA	40	Wagner et al., 1995
NIT3	CCT GTG CTC CAT GCT CCG	40	Wagner et al., 1996
Ntspa712	CGC CTT CGC CAC CGG CCT TCC	50	Daims et al., 2001
Pae997	TCT GGA AAG TTC TCA GCA	0	Amann et al., 1996
Nso1225	CGC CAT TGT ATT ACG TGT GA	35	Mobarry et al., 1996
Nsv443	CCG TGA CCG TTT CGT TCC G	30	Mobarry et al., 1996
PAO651	CCC TCT GCC AAA CTC CAG	35	Crocetti et al., 2000
PAO462	CCG TCA TCT ACW CAG GGT ATT AAC	35	Crocetti et al., 2000
PAO0846	GTTAGCTACGGCACTAAAAGG	35	Crocetti et al., 2000

6.4 RESULTS AND DISCUSSION

6.4.1 Reactor performance evaluation

The SMBR was inoculated with 1.40 g VSS L^{-1} of aerobic sludge obtained from a full-scale STP located in NW Spain. This inoculum had poor settling properties (SVI around 640 mL g^{-1} VSS). During the entire SMBR operation, temperature and pH were not controlled but monitored, being around 19-20 °C and 8.4-8.7, respectively. The oxygen concentration in the SBR unit was maintained above 2 mg L^{-1} during aerobic phases and around 0.1 mg L^{-1} in anoxic stages.

At the end of the 104 days of the start-up period (P1), in which no PPCPs were added to the system, COD, N and P removal efficiencies accounted up to 95%, 71% and 36%, respectively. The biomass in this stage was flocculent with spongy, irregular and bad settling properties (maximum SVI values up to 1000 mL g^{-1} VSS) due to the presence of a variety of filamentous microorganisms. Suspended biomass reached maximum concentration of 2.22 g VSS L^{-1} in the SMBR. After this initial stage, the feeding solution was spiked with the selected pharmaceuticals from concentrated stock solutions (2000 mg L^{-1}) of individual compounds dissolved in methanol (antibiotics, anti-inflammatories and fluoxetine) or acetone (carbamazepine and diazepam). During the following 96 days (P2) N and P removal efficiencies were not affected, while COD removal efficiencies increased up to 98%. However, an increase of biomass concentration was observed, up to 3.30 g VSS L^{-1}, although with only slightly better settling characteristics (SVI around 580 mL g^{-1} VSS). In this period, the most common filamentous bacteria were *Flexibacter* spp. type 1702 which is gram-negative and is responsible for bulking and poor settling properties (Kämpfer and Wagner, 2002). The growth of these bacteria could be attributed to the relative lack of substrate and nutrients in the external filtration chamber, since biological reactions took place preferentially inside the SBR tank. Furthermore, the complete retention of biomass by the membrane and its recirculation into the SBR unit led to poor settling characteristics of biomass, as commonly reported for MBRs (Wang et al., 2010).

From day 202 onwards (P3), SMBR was supplemented with a single addition of PAC at a concentration of 1 g L^{-1} in accordance with previous works (Serrano et al., 2010). Powdered AC was chosen instead of GAC in order to prevent potential erosion of the membrane. PAC addition significantly influenced the performance of the system, leading to increase of COD, N and

P removal efficiencies up to 98%, 81% and 80%, respectively. However, COD removal efficiency was no affected. Taking into account the operating VER, the maximum theoretical N removal would be 78%. The higher values obtained during P3 are due to the higher biomass growth observed, which represented 7.6% of total N removal. Phosphorus removal with activated carbon in activated sludge systems has not been deeply studied. However, a number of media have been tested as adsorbents for phosphorus removal such as sand in subsurface flow constructed reed beds (Arias et al., 2001), dolomite in pilot vertical-flow constructed wetlands (Prochaska and Zouboulis, 2006) and oyster shells in constructed wetland systems (Park and Polprasert, 2008). These works conclude that the high removal efficiencies observed for P is due to adsorption on these media.

A significant increase of biomass concentration was observed during P3 at a constant increasing trend from 3.5 (including 1 g L^{-1} of PAC on day 200) up to 6 g VSS L^{-1} (day 250). Additionally, a significant enhancement in its settling properties (SVI always below 150 mL g^{-1} VSS). Other researchers have reported that activated sludge shows better settling properties after PAC addition, due to a lower compressibility of sludge flocs as well as a lower content of extracellular polymeric substances inside microbial floc (Satyawali and Balakrishnan, 2009; Cecen et al., 2003; Kim et al., 1998). The decrease in filamentous bacteria and the appearance of small and regular flocs are other characteristics that typically enhance the settling properties of sludge. As mentioned, the amount of filamentous bacteria detected in P3 was lower and corresponded mainly to the type 0041, which normally appears in systems with high SRT. The biomass developed after PAC addition not only presented better settling properties, but also led to the development of a cake layer which was easier to remove by physical cleaning, in this way minimizing fouling.

6.4.2 Morphological Observation and FISH Analysis

Particle size distribution is an important parameter in MBRs since it affects the characteristics of the cake formed by the rejected solids, thereby influencing the filtration process (Satyawali and Balakrishnan, 2009). The mean floc diameter (d_{50} based on volume ratios) shows that the floc diameter decreased from 439 μm in P2 to 108 μm in P3. Floc size distribution between 79-1179 μm was observed during P2 whereas at the end of P3 ranged 23-302 μm. This shift in particle size distribution of the aerobic biomass to smaller sizes was associated with the addition of PAC. Li et al. (2005)

observed the same behaviour in the reduction of floc size and particle size distributions after PAC addition in a submerged membrane bioreactor. Other studies suggested that the presence of PAC shift the particle size distribution down to the lower region because of the attached growth of microorganisms onto the surface of PAC (Kim et al., 1998). Furthermore, microscopic observations (Figure 6.2) of the sludge showed remarkable differences in the biomass, especially before and after the addition of PAC in the aeration tank.

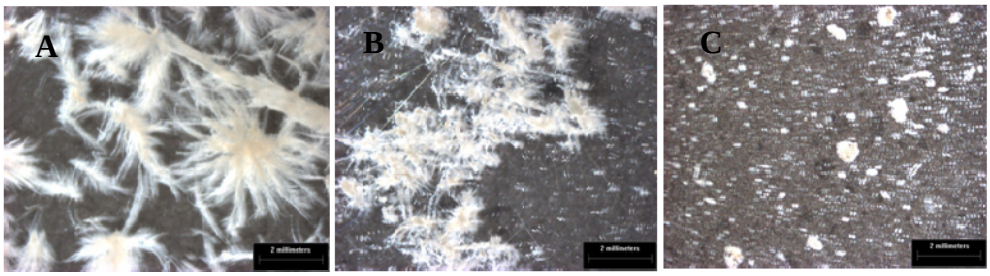


Figure 6.2. Microscopic images of sludge flocs in different days of operation of SMBR: (A) day 76; (B) day 127 and (C) day 259. Scale: 2 mm.

The evolution of microbial populations in the SMBR was followed by FISH analysis. A set of general probes, α-Proteobacteria (ALF1b) and β-Proteobacteria (BET42A), were applied in combination with the general eubacteria domain (EUB3381) to detect the main bacteria involved in the process. In P2, β-Proteobacteria were poorly observed since only few positive results were detected with specific probes for ammonia oxidizing bacteria *Nitrosomonas* (NEU653) and *Accumulibacter* bacteria (PAO651). In the case of α-Proteobacteria, it was evaluated with a specific probe (NIT3) for the identification of nitrite oxidizing bacteria *Nitrobacter* (NOB), but no positive results were detected. Furthermore, denitrifying bacteria *Pseudomonas spp*. (Pae997) gave few positive results. In P3, a much more diverse biocenosis was observed: i) an increase in β-proteobacteria population belonging to halophilic and halotolerant *Nitrosomonas spp*. using probes NEU653 (Figure 6.3); ii) an increase of the positive results were found for Accumulibacter bacteria (Figure 6.3) with different probes (PAO651, PAO0846 and PAO462); iii) few positive results were detected with probes for NOB, NIT3 and Ntspa712, and finally; iv) a higher density of denitrifying bacteria was observed when using probes Pae997. The results indicate a slight enrichment in nitrifying and denitrifying bacteria, which make possible the system to reach the theoretical maximum of nitrogen removal efficiency. Additionally, the detection of PAO indicates that the

enhancement in phosphorus removal during P3 might be attributed not only to sorption onto PAC, but also to the accumulation within the cells of *Accumulibacter* organism as polyphosphate. As previously indicated, the enhancement of biomass-solution contact caused by PAC addition appears to be the key point not only for increasing biomass but also to develop a much more complex and diverse microbial ecology in the system.

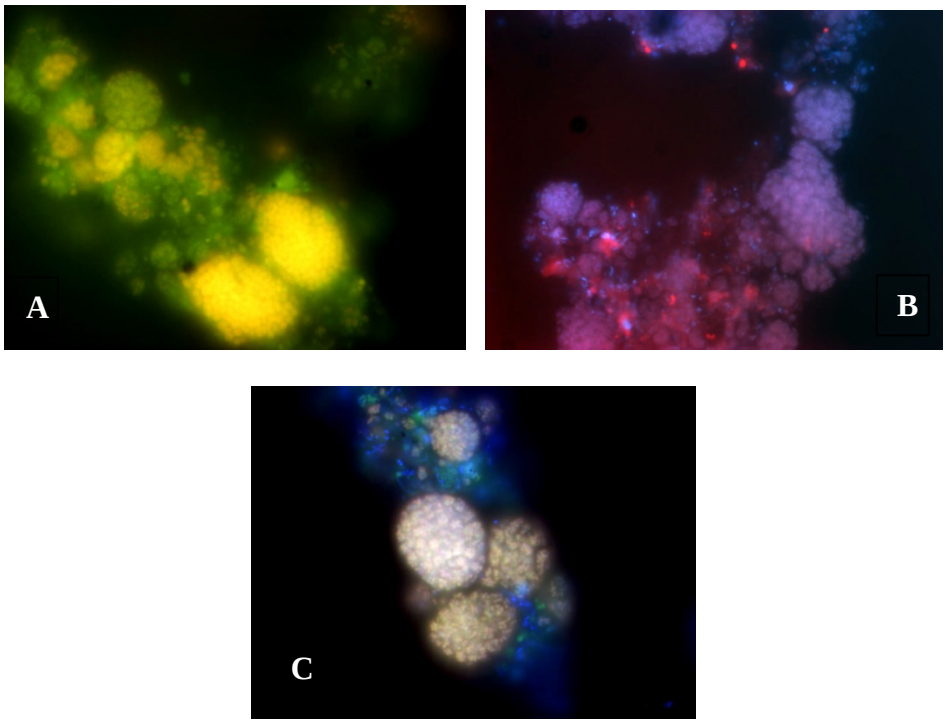


Figure 6.3. FISH images of samples in P3. (A) Ammonia-oxidizing bacteria are marked in orange by superposition of green (NEU653) and red (BET42a); (B) Denitrifying bacteria are marked in pink by superposition of blue (DAPI) and red (Pae997); (C) Polyphosphate-accumulating bacteria are marked in pale yellow by superposition of blue (DAPI), green (EUB338I) and red (PAO462).

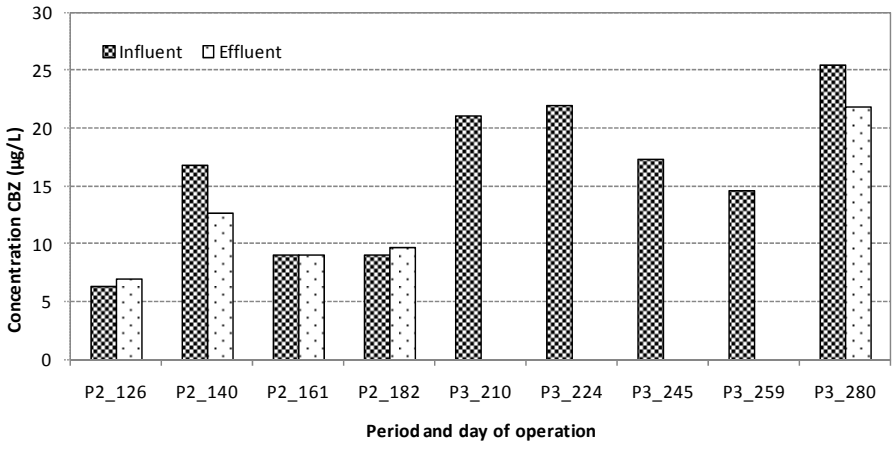
6.4.3 PPCPs Removal

Carbamazepine and Diazepam

Considering the concentration profiles for CBZ and DZP in the liquid phase from the influent to the effluent in P2, no significant removals were obtained

(<20 %). These results are consistent with the low removal efficiencies reported for these compounds when using membrane bioreactors or conventional processes (Radjenovic et al. 2007; Reif et al., 2008; Ying et al. 2009; Suarez et al., 2010) and are related to the low lipophilicity (log K_{ow} 2.3-3.0) and the hardly biodegradable character (k_{biol} < 0.03 L gSS^{-1} d^{-1}) of CBZ and DZP. However, high removal efficiencies of up to 90% during P3 for both compounds were observed, which was associated with the PAC addition. In a previous work (Serrano et al., 2010) it was observed that a single addition of 1 g L^{-1} of GAC into the aeration tank of a CAS system led to removal efficiencies up to 43% for CBZ and around 35% for DZP. Li et al. (2010) also reported that the addition of 1 g L^{-1} of PAC into an MBR treating sewage was a successful tool to remove CBZ up to 92 ±15% during three weeks of operation, indicating that hydrophobicity, loading and PAC dosage were the key factors influencing its removal.

Figure 6.4 shows that the measured removal efficiencies for CBZ and DZP decreased after around three months of operation with PAC (P3). This result is probably due to saturation of the active pores of PAC by organic matter which competes with PPCPs and also by bacteria that grow on PAC. Other studies already pointed out that site competition and pore blockage are two mechanisms involved in the reduction of sorption capacity of target compounds when organic matter is present (Snyder et al. 2007; Fukuhara et al., 2006).



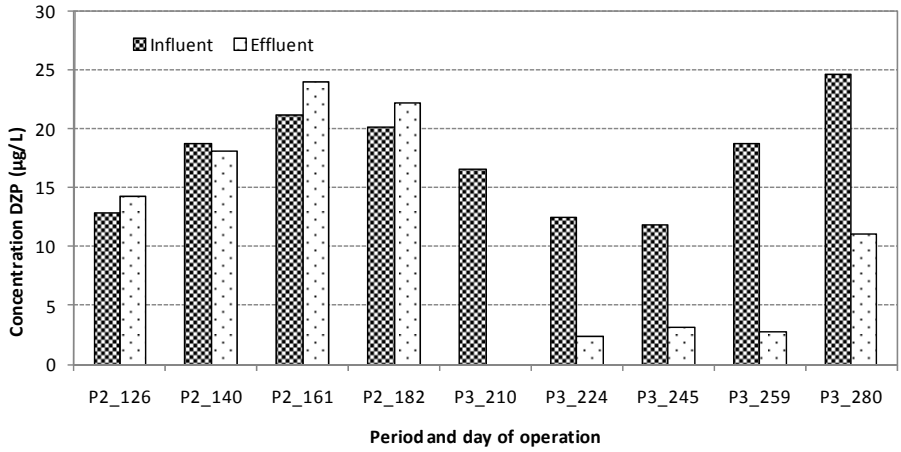


Figure 6.4. Concentrations of CBZ and DZP in the inlet and outlet of SMBR.

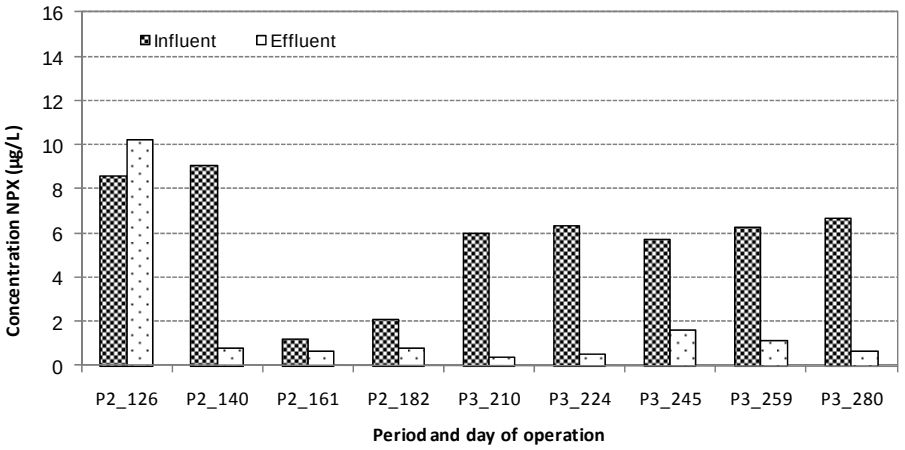
Ibuprofen, Naproxen and Diclofenac

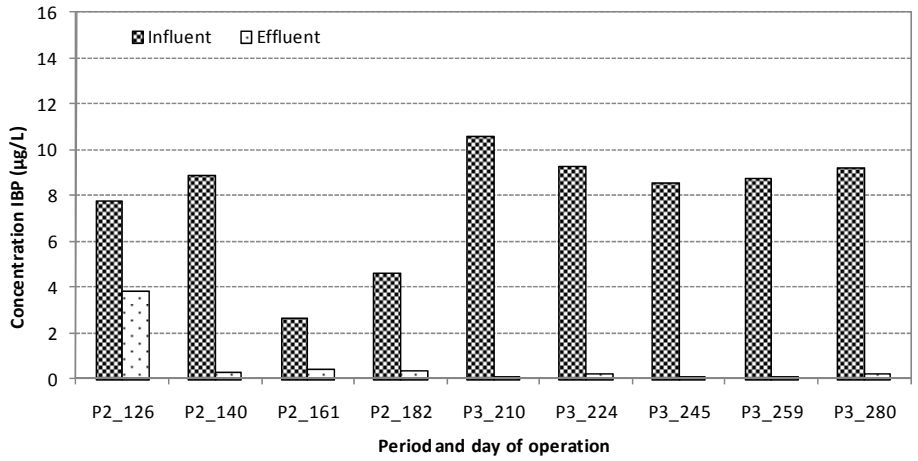
After three weeks of operation with PPCPs, medium removal efficiencies were observed for IBP (51%), which were enhanced to almost complete removals (>90%) after day 140 (Figure 6.5). The high removal of IBP during biological treatment has been confirmed in literature (Suarez et al., 2010; Jones et al., 2007; Kimura et al., 2007). Due to the low affinity for solids, no sorption onto sludge is expected for this compound, being the main removal mechanism biodegradation (k_{biol} 9-35 L g^{-1} SSV d^{-1}).

No removal was observed in the case of NPX during the first three weeks of operation with PPCPs, although similar to IBP, after this initial adaptation period high removal efficiencies were determined (>90%) along P2 and P3 (Figure 6.5). Similarly to IBP, the main removal mechanism for NPX is biodegradation. Other researchers observed that the development a wide microbial diversity, as indicated by the gradual increase of ammonium biodegradation, might be the cause of those increases (Serrano et al., 2010; Suarez et al., 2010; Radjenovic et al. 2007; Kimura et al., 2007). Moreover, no difference in the removal efficiencies was observed for both compounds between P2 and P3, which indicates that PAC has low affinity for these compounds.

Similarly to CBZ and DZP, DCF was not significantly removed in the SMBR during P2, whereas PAC addition caused a very high removal, up to 93%. Unlike CBZ and DZP, for which removal efficiencies in the range of 80-90%

were maintained for two months, in this case this level was only achieved during 1 month approximately (Figure 6.5). These values are in the same range as those obtained previously after the addition of 0.5 g L^{-1} of GAC directly into the aeration tank of a CAS system (Serrano et al., 2010).





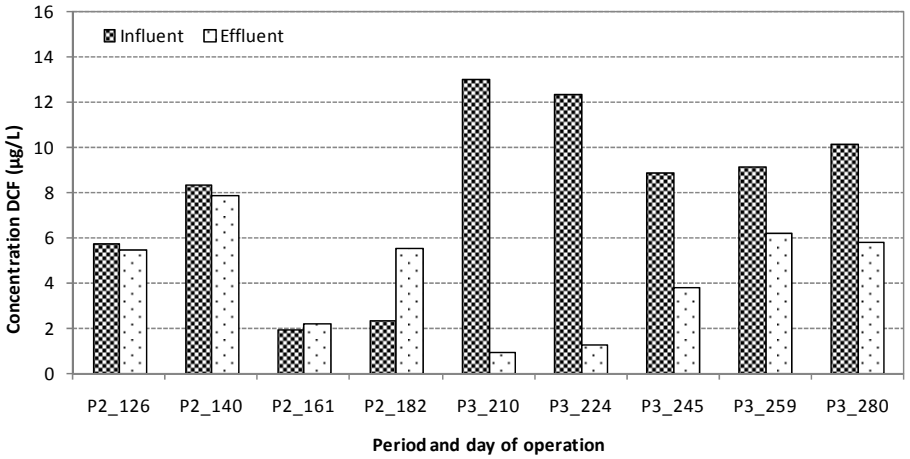


Figure 6.5. Concentrations of IBP, NPX and DCF in the inlet and outlet of SMBR.

Antibiotics

During P2 the three antibiotics used exhibited different behaviour: ROX was highly removed (71-86%), removals obtained for ERY were moderate (42-64%) whereas no significant removal was obtained for TMP. These results are considerably higher than those previously reported in full-scale installations (Göbel et al., 2007; Joss et al., 2005). Suarez et al. (2010) reported high eliminations for ERY and ROX under aerobic conditions (>89%) in a lab-scale CAS reactor, whereas TMP exhibited a recalcitrant behaviour, as indicated in the literature TMP (Castiglioni et al., 2006; Lindberg et al., 2005). Batt et al. (2006) concluded that TMP can be only degraded at a certain extent in activated sludge systems in which nitrifying bacteria are present. In fact, the lower amount of nitrifying bacteria detected using the FISH technique in P2 would explain the lack of removal detected.

On the other hand, PAC addition caused an almost complete removal for the three antibiotics during P3 (Fig. 6.6), which was maintained during more than 2 months. In fact, PAC saturation rate appears to be a very slow process for these substances.

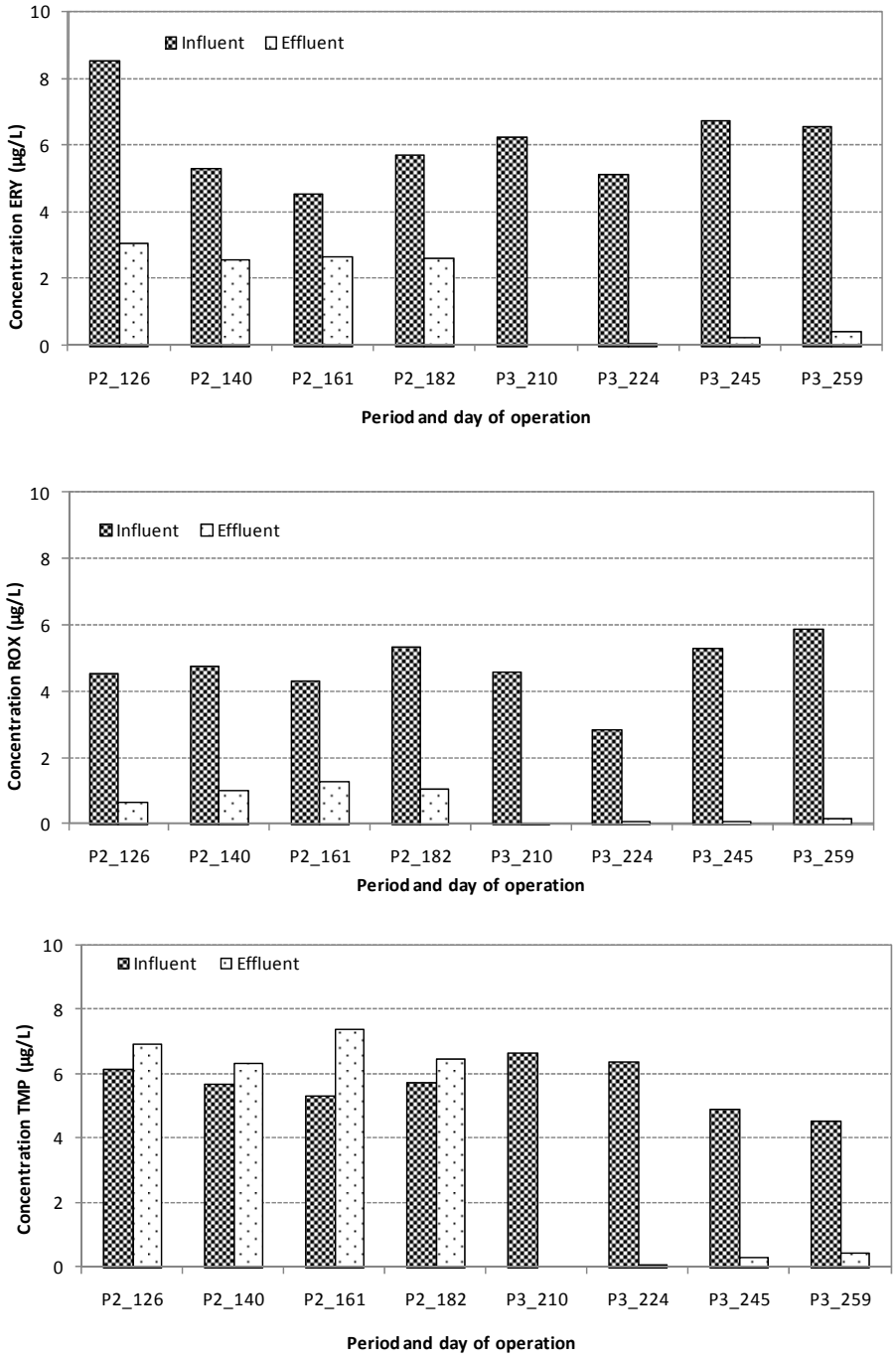


Figure 6.6. Concentrations of ERY, ROX and TMP in the inlet and outlet of SMBR.

Fluoxetine

The operation with the SMBR during P2 has shown to be successful to remove FLX at a high extent, in the range of 82-89%. Similar eliminations were previously reported by Suarez et al. (2010) and Zorita et al. (2009). However, there is a lack of conclusive information about the physico-chemical characteristics of this compound which makes it difficult to draw conclusions about removal mechanisms. According to Kwon and Armbrust (2006), FLX is quite persistent to biodegradation, so it is expected that the removal rates are more related to sorption onto solids. In this sense, the improvement observed during after PAC addition during P3, with removal efficiencies in the liquid phase up to 98%, is very likely the result of the combined interaction between this compound and the biomass and the PAC (Figure 6.7).

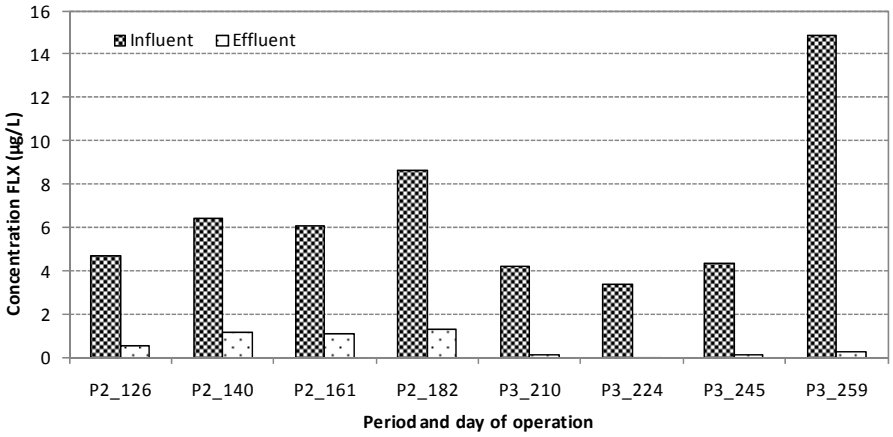


Figure 6.7. Concentrations of FLX in the inlet and outlet of SMBR.

Comparative affinity for activated carbon and saturation

As previously reported, although most of the compounds tested in this work can be significantly removed using activated carbon, their affinity degree for this adsorbent is different. Working with various natural waters, Westerhoff et al. (2005) showed that substances like IBP were poorly adsorbed to PAC (<20%), whereas TMP or FLX were highly removed (>90%). In this work, the highest removal efficiencies obtained after PAC addition corresponded to CBZ, TMP, ROX, ERY and FLX (97-99%). All of these substances have in common a pKa value in the range 7-10, i.e. they are neutral or moderately alkaline. Taking into account that all of these compounds have amino groups

in their structures that will be protonated under neutral conditions forming cations (Babic et al., 2007), it is reasonable to explain the higher affinities exerted by these compounds because of the electrostatic interactions between the cations and the PAC. Other substances like DCF were highly removed after PAC addition, although its maximum removal efficiency was 93%, which was clearly decreasing in the following samples, thus showing a lower PAC affinity. The highest removal efficiency (99%) was achieved for the recalcitrant CBZ, which was maintained along more than two months of operation. In fact, saturation was only observed in the sampling carried out 80 days after PAC addition (CBZ removal efficiency of 14%). Saturation was also observed for other compounds such as DZP and TMP although with different kinetics, probably as the result of their different affinity for PAC and the competition for the available PAC adsorption sites with the other substances present in the mixed liquor.

6.5. CONCLUSIONS

A Sequential Membrane Biological Reactor (SMBR) comprising a SBR unit coupled with an external MF flat-plate membrane was operated to study the removal of selected PPCPs as well as conventional pollutants during the treatment of synthetic municipal wastewaters. After 200 days of operation moderate removals (42-64%) were observed for NPX and ERY, whereas IBP, ROX and FLX were removed in the range of 71-97%. During this period biomass showed poor settling characteristics as well as a low microbial diversity.

After a single addition of 1 g L^{-1} of PAC directly into the aeration tank an immediate and sharp removal increase was observed for the more recalcitrant PPCPs not previously degraded at any significant extent: CBZ, DZP, DCF and TMP, with removal efficiencies in the range of 93-99%. Moreover, other substances which were moderately degraded such as ERY, ROX and FLX were completely removed after PAC addition (97-99%).

The kinetics of saturation of PAC was different for each compound. An almost complete removal was maintained for CBZ along more than two months of operation, being saturation only observed 80 days after PAC addition (CBZ removal efficiency of 14%). Saturation was also observed for other compounds such as DZP and TMP although with different kinetics, probably as the result of their different affinity for PAC and the competition for the available PAC adsorption sites with the other substances present in the mixed liquor.

Biomass characteristics were strongly affected by the presence of PAC. Physico-chemical properties such as conformation of agglomerates and settling were clearly enhanced (more compact flocs, lower SVI) which led to the development of a cake layer which was easier to remove by physical cleaning in this way minimizing fouling. Moreover, microbial diversity was also positively affected, since nitrifiers were detected at higher amounts as well as phosphorus accumulating organisms (PAOs), which improved P removal (up to 80%).

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Conclusiones generales

A continuación se presentan las conclusiones generales de la presente Tesis Doctoral, en la que se aplicaron distintos sistemas de tratamiento de aguas residuales tales como un reactor convencional de lodos activos, un reactor híbrido y un reactor secuencial SBR acoplado a una cámara externas con una membrana, con el objetivo global de estudiar la eficacia de eliminación de una clase de microcontaminantes orgánicos tales como los compuestos farmacéuticos y de cuidado personal (Pharmaceutical and Personal Care Products, PPCPs) presentes en aguas residuales urbanas. Además, en algunos de estos sistemas de reacción biológica se estudió la adición de aditivos tales como coagulantes (cloruro de hierro) y carbón activo (en forma granular o en polvo) directamente al tanque de aireación como una estrategia de maximización de la eliminación de estas sustancias, especialmente aquellas con naturaleza más recalcitrante.

Factores que influyen en la adsorción de los microcontaminantes en el lodo

En una primera parte de este trabajo, se estudió la distribución de estas sustancias entre las fases presentes en un sistema complejo como es el licor de mezcla de los tanques de aireación de los reactores biológicos. Se aplicó una metodología que considera el licor de mezcla como una matriz con tres compartimentos: la fase acuosa, la fase sólida y una tercera fase que comprendería a la materia disuelta y coloidal. Este modelo de tres fases permitirá adquirir un mejor conocimiento sobre el grado de afinidad de una sustancia por cada una de estas fases, determinándose así no tan solo el coeficiente de distribución global sólido-líquido K_d, sino también dos constantes de equilibrio de sorción K_{part} y K_{DCM}, que determinan la afinidad del compuesto hacia las partículas o hacia la materia disuelta y coloidal, respectivamente. En este trabajo, esta metodología fue aplicada para la hormona sintética 17α-etinilestradiol (EE2), determinándose que muestra mayor afinidad hacia las partículas, por el mayor valor obtenido de la constante K_{part} (384 L Kg^{-1}) en comparación con el valor de la constante K_{DCM} (45 L Kg^{-1}). Estos datos proporcionan una mejor información sobre el destino final de esta sustancia en función de las fases presentes en un determinado compartimento ambiental y podría ser aplicada a otros microcontaminantes

para predecir el transporte, destino y comportamiento de estos compuestos en un lodo en función de sus características.

Eliminación de microcontaminantes orgánicos de las aguas residuales urbanas mediante un sistema de lodos activados y un sistema híbrido de lodos activados

La segunda parte del trabajo trata sobre la influencia que sobre la eliminación de los PPCPs pueda tener la presencia de una biopelícula desarrollada sobre un soporte añadido al tanque de aireación de un sistema de lodos activos y fue evaluada utilizando un reactor aerobio híbrido (Hybrid Aerobic System, HAS) durante un periodo de 60 días. Las eficacias de eliminación de macro- y micro-contaminantes en este sistema fueron comparadas con las obtenidas en un sistema convencional de lodos activos (CAS). Pueden observarse dos diferencias significativas entre ambos reactores:

i) En el sistema HAS se obtuvieron eficacias de eliminación de nitrógeno ($N\text{-}NH_4^+$) ligeramente mayores en comparación con el sistema CAS. Esta mayor actividad metabólica también se refrenda por la mayor presencia de bacterias nitrificantes y desnitrificantes en el sistema HAS, determinada mediante el uso de técnicas de biología molecular FISH.

ii) Se observó una mayor eliminación de los antiinflamatorios ibuprofeno y naproxeno, con una eficacia de eliminación muy alta desde el inicio de la operación del sistema HAS.

Por lo tanto, a pesar de la limitada extensión en el tiempo de este trabajo, parece que la presencia de la biopelícula que va colonizando el soporte sintético usado (anillos Kaldnes) puede ejercer un efecto sobre la eliminación de algunos microcontaminantes. Sin embargo, sería preciso continuar este estudio en un sistema híbrido en donde se disponga de una biopelícula suficientemente desarrollado para poder determinar con mayor fiabilidad su posible efecto.

Influencia del empleo de agentes de adsorción y coprecipitación para la eliminación de PPCP en sistemas convencionales de lodos activados (CAS)

En la tercera parte del trabajo se estudió el uso de aditivos como coagulantes (cloruro de hierro) o carbón activo en forma granular (GAC) añadidos directamente al tanque de aireación de reactores convencionales de lodos activos (CAS) como una posible estrategia de mejora en la eliminación de microcontaminantes, especialmente aquellos de carácter más persistente. Los resultados del efecto del uso de ambos aditivos sobre la eliminación de los PPCPs fueron comparados con los resultados obtenidos en un reactor convencional de lodos activos (R1) operado bajo las mismas condiciones. La adición continua de coagulante no mejoró significativamente la eliminación de PPCPs, mientras que con la adición de GAC utilizando las concentraciones más altas (0.5-1 g L^{-1}) se logra mejorar la eficacia de eliminación para algunos PPCPs sobre todo en aquellos con carácter más recalcitrante.

Adicionalmente, se determinaron las isotermas de adsorción de los PPCPs evaluados, obteniéndose que el GAC tiene una mayor afinidad por los compuestos más recalcitrantes (carbamazepina, diazepam y diclofenaco), seguidos de los anti-inflamatorios (ibuprofeno y naproxeno) y por último con las fragancias (tonalide, celestolide y galaxolide).

Según los resultados obtenidos de los microcontaminantes evaluados en los tres reactores y en los ensayos de adsorción, pueden distinguirse los siguientes comportamientos:

i) *Compuestos con comportamiento similar en los tres reactores, indicando baja afinidad por las sales metálicas ó por la presencia GAC*:

   a. Anti-inflamatorios (ibuprofeno y naproxeno) con eliminaciones mayores del 90% desde finales del primer periodo de operación. Ambos compuestos requieren de 100 a 130 días para alcanzar sus máximas eliminaciones. Se puede atribuir a la biodegradación como el principal mecanismo de eliminación de estos compuestos, así como también con la correlación del desarrollo de una biomasa más enriquecida en cada reactor (por ejemplo, presencia de bacterias nitrificantes).

   b. Fragancias (tonalide, celestolide y galaxolide) con eliminaciones del 90% tras 40 días de operación y teniendo eliminaciones casi completas (95-99%) posteriormente a este periodo. Debido a la naturaleza lipofílica de estos compuestos se atribuye que su interacción con los lodos (absorción), es la

principal vía de retención en los reactores promoviendo así su eliminación.

ii) *Compuestos con carácter más recalcitrante y con mayor afinidad por el GAC, atribuyendo su eliminación a un mecanismo de adsorción*:

a. Cabarmazepina, que mejora la eliminación de un 12% a un 43% en presencia de 1 g L^{-1} de GAC.

b. Diazepam, que se elimina hasta un 35% con 1 g L^{-1} de GAC, frente a un 10-25% en un reactor de lodos activos.

c. Diclofenaco, eliminado en un 85% con 0.5 g L^{-1} de GAC, frente a un 75 % en los reactores convencionales.

Eliminación de microcontaminantes persistente de las aguas residuales mediante la adición de carbón activado en polvo a un biorreactor secuencial de membrana

En la última parte del trabajo, se evaluó el uso de carbón activo en polvo (PAC) en un sistema más complejo y con un mayor volumen como es un reactor secuencial tipo SBR acoplado a una membrana externa de microfiltración (Sequential Membrane Biological Reactor, SMBR). Tras la operación con un tiempo de retención de sólidos de 90-100 días (valores a partir de los cuales empieza a observarse saturación del PAC en cuanto a la eliminación de los PPCPs), las características del efluente final del proceso se pueden resumir en: 95-99% de eliminación de materia orgánica, 70-80% de eliminación de $N\text{-}NH_4^+$ y alrededor de 80% de eliminación de $P\text{-}PO_4^{-3}$. En función de la eliminación de los compuestos farmacéuticos considerados se distinguen dos comportamientos:

i) *Compuestos con mayor afinidad por el PAC y que no son eliminados de forma significativa en ausencia de éste*: carbamazepina, trimetoprim, roxitromicina, eritromicina y fluoxetina, obteniéndose eliminaciones muy altas (97-99%). Las características principales que tienen en común estos compuestos es que todos ellos tienen un pKa entre 7-10 y todos ellos tienen grupos aminos en sus estructuras moleculares, lo que puede ocasionar que puedan estar parcialmente protonados en condiciones neutras. El principal mecanismo de eliminación de

estos compuestos radica en su interacción físico-químico con el PAC.

ii) *Compuestos de biodegradabilidad media como ibuprofeno y naproxeno*. Estos compuestos presentan altos porcentajes de eliminación (> 90%) siendo la biodegradación el principal mecanismo de eliminación, mientras que la adsorción en lodos o en el PAC no es significativa. Sin embargo, la mayor abundancia de organismos nitrificantes observada tras la adición de PAC mejora aún más su eliminación. El alto tiempo de retención celular del proceso y el desarrollo de una ecología microbiana diversa son los principales factores que explicarían la mejora en la eliminación de estas sustancias.

Los resultados obtenidos en este estudio permiten alcanzar una visión clara sobre el efecto del empleo de un adsorbente directamente añadido en un proceso biológico, en este caso carbón activo ya sea en polvo o granular y comprobar que es una buena estrategia para mejorar la eliminación de microcontaminantes orgánicos, especialmente de aquellos compuestos que se pueden adsorber con facilidad y que tengan una biodegradabilidad baja. En función de los buenos resultados obtenidos en este trabajo, se sugiere que el empleo de estas configuraciones podría no solo resultar interesante en el tratamiento de aguas residuales urbanas sino también para aquellos efluentes que puedan tener altas concentraciones de PPCPs tales como los efluentes de hospitales, industrias farmacéuticas o explotaciones agropecuarias que empleen medicamentos veterinarios.

Conclusións xerais

A continuación preséntanse as conclusións xerais da presente Tese Doutoral, na cal se aplicaron distintos sistemas de tratamento de augas residuais tales como reactores convencionais de lodos activos, reactores híbridos e reactores secuenciais SBR axustados a cámaras externas de membrana, co obxectivo global de estudar a eficacia de eliminación dunha clase de microcontaminantes orgánicos tales como son os compostos farmacéuticos e de coidado persoal (Pharmaceutical and Persoal Care Products, PPCPs) presentes en augas residuais urbanas. Ademais, nalgúns destes sistemas de reacción biolóxica estudouse a adición de aditivos tales como coagulantes (cloruro de ferro) e carbón activo (en forma granular ou en po) directamente dentro do tanque de aireación como unha estratexia de maximización da eliminación destas sustancias, especialmente aquelas con natureza máis recalcitrante.

Factores que inflúen na absorción dos microcontaminantes no lodo

Nunha primeira parte deste traballo, estudouse a distribución destas sustancias entre as fases presentes nun sistema complexo como é o licor de mestura dos tanques de aireación dos reactores biolóxicos. Aplicouse unha nova metodoloxía que considera este licor de mestura como unha matriz con tres compartimentos: a fase acuosa, a fase sólida e unha terceira fase que comprendería á materia disolta e coloidal. Este modelo de tres fases permitiría ter un maior coñecemento sobre o grao de afinidade que ten unha sustancia por cada unha destas fases, determinándose así non só o coeficiente de distribución global sólido-líquido K_d, senón tamén as dúas constantes de equilibrio de sorción K_{part} e K_{DCM}, que determinan a afinidade do composto cara ás partículas ou cara á materia disolta e coloidal, respectivamente. Neste traballo, esta metodoloxía foi aplicada para a hormona sintética 17α-etinilestradiol (EE2), determinándose que a maior afinidade que mostra esta sustancia é cara ás partículas de acordo ao valor obtido para a constante K_{part} (384 L Kg^{-1}) en comparación co valor da constante K_{DCM} (45 L Kg^{-1}). Estes datos ofrécennos maior información sobre o destino final desta sustancia en función das fases presentes nun determinado compartimento ambiental e poderían ser aplicados a outros

microcontaminantes para predicir o transporte, destino e comportamento destes compostos nun lodo en función dás súas características.

Eliminación de microcontaminantes orgánicos das augas residuais urbanas mediante un sistema de lodos activados e un sistema híbrido de lodos activados

A segunda parte do traballo trata sobre a influenza que sobre a eliminación dos PPCPs poida ter a presenza dunha biopelícula desenvolvida sobre un soporte sintético engadido ao tanque de aireación dun sistema de lodos activos. Isto foi avaliado utilizando un reactor aerobio híbrido (Hybrid Aerobic System, HAS) durante un período de 60 días. As eficacias de eliminación de macro- e micro-contaminantes neste sistema foron comparadas coas obtidas nun sistema convencional de lodos activos (CAS). A pesar do pouco tempo de avaliación conxunta de ámbolos dous sistemas, poden sinalarse dúas diferenzas significativas entre os reactores:

i) Non sistema HAS obtivéronse eficacias de eliminación de nitróxeno (N- NH_4^+) lixeiramente maiores en comparación co sistema CAS. Esta maior actividade metabólica tamén se confirma pola maior detección de bacterias nitrificantes e desnitrificantes obtida no sistema HAS mediante o uso de técnicas de bioloxía molecular como a denominada FISH.

ii) Observouse unha maior eliminación de dous microcontaminantes, os antiinflamatorios ibuprofeno e naproxeno, os cales mostran unha eficacia de eliminación moi alta desde o inicio da operación do sistema HAS.

Polo tanto, a pesar das limitacións deste traballo (reducido tempo de operación do reactor HAS) parece que a presenza da biopelícula que vai colonizando o soporte sintético usado (aneis Kaldnes) logra ter un mellor efecto sobre a eliminación dalgúns microcontaminantes. Con todo, sería preciso continuar este estudo nun sistema híbrido onde se dispoña dunha biopelícula suficientemente desenvolvida para poder determinar con maior fiabilidade a súa posible influencia na eliminación de PPCPs.

Influencia do emprego de axentes de absorción e coprecipitación para a eliminación de PPCP en sistemas convencionais de lodos activados (CAS)

Na terceira parte do traballo estudouse o uso de aditivos como coagulantes (cloruro de ferro) ou carbón activo en forma granular (GAC) engadidos directamente dentro do tanque de aireación de reactores convencionais de lodos activos (CAS) como unha estratexia de mellora da eliminación de microcontaminantes, especialmente aqueles de carácter máis persistente. Os resultados do efecto do uso de ámbolos dous aditivos sobre a eliminación dos PPCPs foron comparados cos resultados obtidos nun reactor convencional de lodos activos (R1) baixo as mesmas condicións que os outros reactores. A adición continua de coagulante dentro do tanque de aireación dun dos reactores (R2) non mellorou significativamente a eliminación de PPCPs, mentres que coa adición de GAC dentro do tanque de aireación do reactor R3, utilizando as concentracións máis altas (500-1000 mg L^{-1}), lógranse eficacias de eliminación maiores para algúns PPCPs, sobre todo naqueles con carácter máis recalcitrante.

Adicionalmente, realizáronse ensaios de adsorción determinándose as isotermas dos PPCPs avaliados, obténdose que o GAC ten maior afinidade cos compostos máis recalcitrantes (carbamazepina, diazepam e diclofenaco), seguidos dos anti-inflamatorios (ibuprofeno e naproxeno) e por último coas fragrancias (tonalide, celestolide e galaxolide).

De acordo cos resultados obtidos dos microcontaminantes avaliados nos tres reactores e nos ensaios de adsorción, podemos distinguir os seguintes comportamentos:

i) Compostos con comportamento similar nos tres reactores, indicando baixa afinidade polos sales metálicos ou pola presenza de GAC:

   a. Anti-inflamatorios (ibuprofeno e naproxeno) con eliminacións maiores do 90% desde finais do primeiro período de operación. Ámbolos dous compostos requiren de 100 a 130 días para alcanzar as súas máximas eliminacións. De acordo ás súas propiedades físico-químicas pódese atribuír á biodegradación como o principal mecanismo de eliminación destes compostos, así como tamén coa correlación do desenvolvemento dunha biomasa máis enriquecida en cada reactor (por exemplo, presenza de bacterias nitrificantes).

   b. Fragrancias (tonalide, celestolide e galaxolide) con eliminacións do 90% despois de 40 días de operación e tendo eliminacións case completas (95-99%) despois deste período. De acordo á

natureza hidrofóbica destes compostos considérase que a súa interacción cos lodos (adsorción) é a principal vía da súa retención nos reactores promovendo así a súa eliminación.

ii) Compostos con carácter máis recalcitrante e con maior afinidade polo GAC, atribuíndo a súa eliminación a un mecanismo de adsorción:

   a. Carbamazepina con eliminacións <12% en R1 e R2 e que coa presenza de GAC en concentracións de 1000 mg L^{-1} en R3, lógrase eliminar ate un 43%.

   b. Diazepam con eliminacións do 10 ao 25% en R1 e R2 e con GAC en R3 en concentracións de 500 e 1000 mg L^{-1}, lógrase eliminar 35% e 32%, respectivamente.

   c. Diclofenaco con eliminacións do 74% (R1) e do 70% (R2). No período de avaliación do GAC cando R3 contén unha concentración de 500 mg L^{-1} alcanza a súa máxima eliminación do 85%.

Eliminación de microcontaminantes persistente das augas residuais mediante a adición de carbón activado en po a un biorreactor secuencial de membrana

Na última parte do traballo, avaliouse ou uso de carbón activo en po (PAC) dentro dun sistema máis complexo e cun maior volume como é un reactor secuencial tipo SBR axustado a unha membrana externa de ultrafiltración (Sequential Membrane Biological Reactor, SMBR). Trala operación cun tempo de retención de sólidos de 90-100 días (valores a partir dos cales empeza a observarse saturación do PAC en canto á eliminación dos PPCPs), as características do efluente final do proceso pódense resumir en: 95-99% de eliminación de materia orgánica, 70-80% de eliminación de $N\text{-}NH_4^+$ e ao redor de 80% de eliminación de $P\text{-}PO_4^{-3}$. De acordo á eliminación dos compostos farmacéuticos considerados distinguíronse dous grupos en canto ao seu comportamento:

i) Compostos con maior afinidade polo PAC e que non son eliminados de forma significativa en ausencia deste: carbamazepina, trimetoprim, roxitromicina, eritromicina e fluoxetina, obténdose eliminacións moi altas (97-99%). As características principais que teñen en común estes compostos é que todos eles teñen un pKa entre 7-10 e por tanto poden ser moderadamente alcalinos. Á súa vez estes

compostos teñen a presenza de grupos amino dentro das súas estruturas moleculares o que pode ocasionar que estean parcialmente protonados baixo condicións neutras. Por iso, o principal mecanismo de eliminación destes compostos no proceso radica na súa interacción co PAC.

ii) Compostos de biodegradabilidade media como o ibuprofeno e o naproxeno. Estes compostos presentan altas porcentaxes de eliminación (> 90%) sendo a biodegradación o principal mecanismo de eliminación, mentres que a absorción en lodos ou no PAC non é significativa. Con todo, a maior abundancia de organismos nitrificantes observada trala adición de PAC mellora aínda máis a súa eliminación. O alto tempo de retención celular do proceso e o desenvolvemento dunha ecoloxía microbiana diversa son os principais factores que explicarían a eliminación destas sustancias.

Os resultados obtidos neste estudo serven para ter unha visión clara de que o emprego dun adsorbente, neste caso carbón activo xa sexa en po ou granular, é unha boa estratexia para mellorar a eliminación de microcontaminantes orgánicos, especialmente tendo un maior efecto naqueles compostos con carácter máis recalcitrante. De acordo aos bos resultados obtidos neste traballo, é posible suxerir que o emprego do adsorbente pode ser aplicado ao tratamento de distintas augas residuais que poden proceder de hospitais, industrias farmacéuticas e/ou explotacións agropecuarias que empreguen medicamentos veterinarios.

General conclusions

In this work, different wastewater treatments, such as conventional activated sludge reactor, hybrid reactor and Sequential Batch Reactor with an external microfiltration membrane, were applied to study the removal of specific organic micropollutants such as PPCPs present in urban wastewater. Moreover, the addition of some additives such as coagulants (Ferric Chloride) and activated carbon (either in granular or powdered form) directly to the aeration tank of the conventional activated sludge system were studied, as a strategy to enhance PPCPs removal, particularly for compounds with a recalcitrant behaviour.

Factors influencing adsorption of micropollutants on sludge

In the first part of this work, micropollutants distribution between the phases present in the aeration tank was studied by using a methodology that considers sludge as a three compartment matrix: aqueous, solid and a third phase that includes dissolved and colloidal matter. This model permits to gain a better knowledge about the affinity of the pollutant to every phase, through the determination not only of the solid-water distribution coefficient, but also of two equilibrium constants: K_{part} and K_{DCM}, which describe the compound affinity to particles or dissolved and colloidal matter, respectively. In this study, this methodology was applied to the synthetic hormone 17α-ethinylestradiol (EE2). The results show that EE2 displays a higher affinity to particulate matter, according to the value of K_{part} (384 L Kg^{-1}) compared to the value of the K_{DCM} constant (45 L Kg^{-1}). This methodology cand be applied to other micropollutants to predict their transport, fate and behaviour in a sludge as a function of its characteristics.

Removal of organic micropollutants from urban wastewater by an activated sludge system and hybrid activated sludge process

The second part was focused on the influence of the biofilm developed on a synthetic carrier in the aeration tank on the removal of PPCPs. This evaluation was carried out in a hybrid aerobic system during a period of 60

days. The macro- and micro- pollutants removal efficiencies were compared with the results obtained in a conventional activated sludge system. Two differences between both reactors can be drawn:

i) HAS system shows a slightly higher nitrogen ($N\text{-}NH_4^+$) removal in comparison with CAS system. This higher metabolic capacity was confirmed by the detection of some groups of bacteria such as ammonia oxidizers and denitrifiers in HAS reactor by means of a FISH technique.

ii) High removal efficiencies of two micropollutants were observed, the anti-inflammatories ibuprofen and naproxen, which showed higher removal efficiencies since the beginning of the operation of HAS system.

Therefore, despite of the short operation time of HAS reactor, it seems that the presence of the biofilm that colonized the carrier (Kaldnes carrier) allowed to achieve a better effect on the removal of some micropollutants. However, it would be of interest following a hybrid system with well-developed biofilm in order to determine its real influence on PPCPs removal.

Influence of the employment of adsorption and coprecipitation agents for the removal of PPCPs in Conventional Activated Sludge (CAS) systems

In a third part, the use of additives such as coagulant Ferric chloride or Granular Activated Carbon (GAC) added directly to the aeration tank of a conventional activated sludge system as a strategy to improve removal of micropollutants, mainly for compounds with recalcitrant behaviour, was studied. The results obtained by the addition of these additives were compared with the removal efficiency of micropollutants in a conventional activated sludge system (R1) under the same operational conditions. The continuous addition of a coagulant to reactor R2 did not enhance significantly PPCPs removal, while the addition of GAC directly into the aeration tank of R3 at the highest concentrations (0.5-1 g L^{-1}) allows to improve removal efficiencies of the more recalcitrant compounds.

In addition, batch assays were carried out to determine the adsorption isotherms of the PPCPs studied. GAC had a higher affinity to the recalcitrant compounds (carbamazepine, diazepam and diclofenac), following to the anti-

inflammatory compounds (ibuprofen and naproxen) and finally for musk fragrances (tonalide, celestolide and galaxolide).

i) According to the results obtained for PPCPs removal in the three reactors and batch assays, two types of behaviour were observed: *Compounds with similar behaviour in the three reactors, which reported low affinity to metallic salts or the presence of GAC:*

   a. Anti-inflammatories (ibuprofen and naproxen) achieve high removal efficiencies (>90%) when system is mature enough (after 100-130 d). Considering their physico-chemical properties, it can be concluded that biodegradation is the main removal mechanism of these compounds.

   b. Musk fragrances (tonalide, celestolide and galaxolide) with removal efficiencies of 90% after 40 days of operation in each reactor which later increase, reaching almost complete removal (95-99%). Taking into account the lipophilic nature of these compounds, the sorption onto sludge (absorption) is assumed to be the main removal mechanism.

ii) *Compounds with more recalcitrant behaviour and a positive effect of GAC on removal*:

   a. Carbamazepine, which efficiencies removal increases from 12% to 43% in presence of 1 g L^{-1} of GAC.

   b. Diazepam, with enhanced removal from 10-25% to 30-35% by addition of 0.5-1 g L^{-1}.

   c. Maximum removal efficiencies for Diclofenac (85%) obtained when the concentration of GAC was 0.5 g L^{-1} against 70%.

Removal of persistent pharmaceutical micropollutants from sewage by addition of PAC in sequential membrane bioreactor

In the last part of this work, the use of Powdered Activated Carbon (PAC) was evaluated in a Sequential Membrane Biological Reactor (SMBR) with an microfiltration membrane. When working at 90-100 days of sludge retention time in the system (once saturation of PAC was achieved), the final characteristics of the effluent in the system attain a 95-99% of organic

matter removal, 70-80% of $N\text{-}NH_4^+$ removal and 80% of $P\text{-}PO_4^{-3}$ removal. Two types of behaviour have been identified:

i) *Compounds having a higher affinity to PAC and with a negligible removal without the presence of PAC*: high removals (97-99%) of carbamazepine, trimetoprim, roxitromicin, eritromicin and fluoxetine were achieved. All of these compounds have a pKa value in the range of 7-10 and all them contain amino groups in their molecular structures. The main removal mechanism of these compounds is the interaction with the PAC.

ii) *Compounds with limited biodegradability, such as ibuprofen and naproxen*. These compounds present high removal efficiencies (>90%) being biodegradation the main removal mechanism, while adsorption on sludge or PAC is negligible. However, addition of PAC enhanced the development of nitrifying bacteria which allow the efficiency to be increased. Thus, high sludge retention time and the development of a more diverse microbial population ecology explain the increased removal of these substances.

The results obtained in this study confirm that the use of the adsorbent directly in a biological process -in this case using activated carbon either in granular of powdered form- may be a good strategy to enhance removal of organic micropollutants, particularly in the case of the compounds with more recalcitrant behaviour. According to the results obtained in this Doctoral Thesis that configuration is suggested not only for urban wastewater treatment but also for effluents with high PPCPs concentrations, such as hospital effluents, pharmaceutical industries or livestock farms that employ veterinary medicines.

1. List of Acronyms

AC Activated Carbon
AOB Ammonia Oxidizing Bacteria
ASE Accelerated Solvent Extractor
BSA Bovine Serum Albumin
CAS Conventional Activated Sludge
CBZ Carbamazepine
CEL Celestolide
DCF Diclofenac
DCM Dissolved and Colloidal Matter
DOM Dissolved Organic Matter
DZP Diazepam
EDCs Endocrine Disrupting Compounds
EE2 17 α-Ethinylestradiol
FISH Fluorescent In Situ Hybridization
FLX Fluoxetine
GAC Granular Activated Carbon
GLX Galaxolide
HAS Hybrid Aerobic System
HMBR Hybrid Membrane Biological Reactor
HPLC High-Performance Liquid Chromatography
HS Hybrid Systems
IBP Ibuprofen
LC Liquid Chromatography
LOEC Lowest Observed Effect Concentrations
MBRs Membrane Bioreactors
MBBR Moving Bed Biofilm Reactor
MS Mass Spectrometry
NOEC No Observable Effect Concentrations
NOB Nitrite Oxidizing Bacteria
NSAID Nonsteroidal Anti-Inflammatory Drugs
NPX Naproxen
PAC Powdered Activated Carbon
PAHs Polycyclic Aromatic Hydrocarbons
PHB Poly-Hydroxy-Butyrate
PAO Polyphosphate Accumulating Organisms
ROX Roxithromycin
PPCPs Pharmaceutical and Personal Care Products
R Reactor
SMBR Sequential Membrane Batch Reactor
SMX Sulphamethoxazole
STPs Sewage Treatment Plants
SBR Sequencing Batch Reactor
SPE Solid Phase Extraction
SSRIs Serotonin Reuptake Inhibitors
TC Total Carbon
TIC Total Inorganic Carbon
TMP Trimethoprim
TOC Total Organic Carbon
TON Tonalide
TSS Total Suspend Solids
VER Volume Exchange Ratio
VSS Volatile Suspend Solids
WWTP Wastewater Treatment Plant

2. List of Symbols

C_{aqu}	Aqueous phase concentration (sum of the freely dissolved and sorbed-to-DCM states)	mg L^{-1}
C_{DCM}	Sorbed-to-DCM concentration	mg kg^{-1}_{DCM}
$C_{dissolved}$	Dissolved concentration of the substance	g L^{-1}
C_{free}	Freely dissolved concentration	mg L^{-1}
C_{part}	Sorbed-to-particles concentration	mg kg^{-1}_{part}
C_{sorbed}	Sorbed concentration of the substance	g L^{-1}
C_{tot}	Total concentration	mg L^{-1}
COD	Chemical Oxygen Demand	g L^{-1}
COD_T	Total Chemical Oxygen Demand	g L^{-1}
CODs	Soluble Chemical Oxygen Demand	g L^{-1}
DO	Dissolved Oxygen concentration	g O_2 L^{-1}
H	Henry coefficient	µg m^{-3} air µg $^{-1}$ m^{-3} wastewater
HRT	Hydraulic Retention Time	d
IC	Inorganic Carbon	g L^{-1}
K_{biol}	Pseudo first order degradation constant	L kg^{-1}SS d^{-1}
K_d	Sludge-water distribution coefficient	L kg^{-1}
K_{DCM}	Equilibrium constant of sorption of DCM	L kg^{-1}_{DCM}
K_f	Freundlich adsorption coefficient	L kg^{-1}
K_{ow}	Octanol-water partition coefficient	
K_{part}	Equilibrium constant of micropollutant sorption to particles	L kg^{-1}_{part}
NLR	Nitrogen Loading Rate	g N L^{-1} d^{-1}
OLR	Organic Loading Rate	g COD L-1 d^{-1}
pKa	Dissociation constant	
R_{biol}	Biological degradation rate	
SRT	Solids Retention Time	d
SS	Suspended Solids	g L^{-1}
SVI	Sludge Volume Index	mL $(g\ VSS)^{-1}$
T	Temperature	°C
TOC	Total Organic Carbon	g L^{-1}
V	Volume	L
V_{Sludge}	Sludge volume	mL
$V_{Supernatant}$	Supernatant volume	mL
V_{Water}	Water volume	mL
VSS	Volatile Suspended Solids	g L^{-1}
$C_{micropollutants}$	Micropollutant concentration	mg L^{-1}

List of Publications

1. Journal publications

- Serrano, D., Lema, J.M., Omil, F., 2010. Influence of the employment of adsorption and coprecipitation agents for the removal of PPCPs in conventional activated sludge (CAS) system. *Water Science and Technology* 62(3), 728-735.

- Serrano, D., Suárez, S., Lema, J.M., Omil, F., 2011. Removal of persistent pharmaceutical micropollutants from sewage by addition of PAC in a sequential membrane bioreactor. *Water Research*. In press.

2. Journal in preparation

- Serrano, D., Lema, J.M., Omil, F., Patureau, D., Application of a three-compartment model for 17α-ethinylestradiol as a tool to predict its sorption onto sludge. Submitted to *Water, Air and Soil Pollution.*

3. Patent

- D. Serrano, S. Suárez, J.M. Lema, F. Omil. Proceso para la eliminación de productos Farmacéuticos presentes en aguas residuales. *Patent applied for in May 2011* (OEPM Madrid, Spain, nº P201130652).

4. Book chapters

- Suárez, S., Carballa, M., Reif, R., Serrano, D., Lema, J.M., Omil, F., 2010. Mass Balances of Organic Micropollutants in Sewage Treatment Plants. Innovative Technologies for Urban Wastewater Treatment Plants. 191-216. ISBN: 978-84-693-3992-3.

5. Contribution to congress

- Serrano, D., Lema, J.M., Omil, F. Influence of the employment of coprecipitation and adsorption agents for the removal of PPCPs in Conventional Activated Sludge (CAS) systems. Oral presentation. International Conference on Xenobiotics in the Urban Water Cycle-XENOWAC 2009. Paphos (Chipre) on 11-13 march 2009.

- Serrano, D., Lutchmiah, K., Omil, F., Lema, J.M. Removal of organic micropollutants from urban wastewater by an activated sludge system and hybrid activated sludge process. Poster presentation. VII ANQUE International Congress. Integral Water Cycle: Present and Future. Oviedo (Spain) on 13-15 June 2010.

- Serrano, D., Lema, J.M., Omil, F. Removal of persistent pharmaceutical micropollutants from sewage by addition of PAC in sequential membrane bioreactor. Oral presentation. IWA MTWR 2010 Regional Conference and Exhibition on Membrane Technology and Water Reuse. Istambul (Turkey) on 18 -22 October 2010.